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MULTIDISCIPLINARY LABORATORY
(To Study Early Markers of HIV Infection)

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MRI	Contract with private sector

(1) Statement of the problem under study.

To provide a better understanding of the natural history and pathogenesis of neurologic syndromes related to HIV infection, HIV isolation and antibody studies are being performed from blood and CSF of seropositive patients in various stages of disease development ranging from asymptomatic to ARC to AIDS.

(2) Background and review of appropriate literature.

AND

(3) Rationale.

HIV is a pathogenic retrovirus which causes a progressive deterioration of the immune system and is associated with a wide variety of human diseases. Neurological disease is a major problem in the HIV infected population. The purpose of this proposal is to understand the natural history of HIV associated neurologic disease.

Opportunistic infections [1,2,3,4] and tumors [4,5] of the central nervous system (CNS) were the first neurologic diseases reported in HIV infected patients. Subsequently, the most common neurologic manifestation of HIV was found to be a subacute encephalopathy characterized by cognitive deficits, motor slowing, and behavioral changes, called the "AIDS Dementia Complex (ADC)" [6,7,8]. The spectrum of ADC ranges from mild cognitive abnormalities on neuropsychologic testing in otherwise asymptomatic individuals [9] to a bedridden state marked by global dementia, severe hypokinesis, mutism, incontinence, and seizures [6,7,8]. To this date, only HIV has been consistently implicated as the etiologic agent in ADC [9a]. Other neurologic manifestations of HIV include: peripheral neuropathies [10,11, vacuolar myelopathy [12,13], myopathy [14,15] and organic psychosis [8]. It is not known if these are related to ADC or even occur by similar mechanisms.

Recent data, including previous work from our laboratory [16] and our work in progress, have confirmed the neurotropism of HIV; this includes early penetration of the CNS shortly after systemic infection and the now well confirmed finding that a high proportion of HIV infected individuals, including asymptomatics, display cerebrospinal fluid (CSF) abnormalities suggestive of intra-blood-brain-barrier (I-BBB) CNS inflammation [17, see Work Accomplished]. The relationship of these CSF changes to clinical neurologic disease is not clear; to date, no study has confirmed a CSF (or serum) marker for HIV neurologic disease [17,18] and routine CSF abnormalities seem to be equally distributed among neurologically symptomatic and asymptomatic subjects [18, see Work Accomplished].

Preliminary evidence suggests that the presence of CSF p24 antigen (a core viral protein) has been associated with encephalopathy in children [19] and some adults [20] and may be a marker for neurologic disease. It has been proposed that concurrent loss of anti-p24 antibodies in CSF may also be associated with systemic [21] and neurologic progression [22].

Serum HIV p24 antigen can be detected early and transiently during HIV infection. Antibody production follows, after which the presence of p24 antigen usually disappears. The persistence or reappearance of HIV antigenemia in the serum appears to correlate with clinical, immunological, and neurological deterioration [20,23,24]. In a recent study, HIV antigenemia in sera appeared to be a better predictive marker of HIV related complications than the absolute T4 count [23]. In a similar study, levels of core antigen, antibodies to HIV, and numbers of T4 cells were followed as predictors of AIDS [25]. The inability to detect anti-p24 and reverse transcriptase antibody bands by Western Blot has been associated with poor prognosis [26,27,28].

Another marker which has been associated with the evolution of seropositive states to AIDS is beta-2 microglobulin [29,30]. Elevated beta-2 microglobulin levels in CSF have been reported to be associated with the presence and severity of CNS disease [31].

One hypothesis is that CNS dysfunction parallels the decline in systemic immunologic integrity [31] which can be measured by increasing numbers of opportunistic infections and progression in the stage of systemic disease, decrease in T4 cell count and T4/T8 ratio, elevated levels of p24 and other products of virologic replication, and loss of anti-viral antibodies. Other popular theories are: (1) Certain strains of HIV are especially neurotropic or neurovirulent [32]. (2) Replicating HIV may release factors which are toxic to the nervous system; for

example, excess gp 120 produced by replicating HIV may act as a false transmitter [33,34,35] or prevent the uptake of trophic factors necessary for neuronal function [36,37]. (3) Other viruses such as CMV or herpes simplex type 1 may co-infect the CNS and possibly act as enhancers for HIV neuro-disease progression [38,39].

Early viral penetration of the CNS and subsequent CSF abnormalities in early disease have been reconfirmed by us in a very well characterized population. Our preliminary results (see "Work Accomplished") suggest an association between increasing systemic disease, progressive immune dysfunction, and neurologic dysfunction in the absence of opportunistic infection. These initial cross-sectional results do not explain the occurrence of neurologic deficts in some relatively "early" individuals with normal immune system laboratory measures, or the relative preservation of CNS function in some AIDS individuals. It is here that continuation of our longitudinal study, comparing the neurologic status of individuals with changes in their immunologic and virologic parameters, (molecular and biologic characteristics of HIV and their evolution over time) is necessary to elucidate the markers for and possible mechanisms of HIV CNS disease. The longitudinal data and newly proposed laboratory investigations may help to determine whether it is primarily the involution of the immune system, or the biologic characteristics of the invading virus, or some combination of the two which most determine the onset and progression of CNS disease.

Since not all subjects with CSF abnormalities manifest clinical or behavioral changes, CNS penetration alone cannot predict onset of clinical disease. If neurologic function parallel systemic immune changes, this might account for the low incidence of neurologic abnormalities in Marshall's study [18], which was heavily skewed to early disease. However, without baseline immune parameters or sequential followup it may be difficult to prove this hypothesis. Alternatively, some investigators have suggested that the biological variability of HIV has resulted in the appearance of strains with varying degrees of neurotropism and neurovirulence [40,41]. It would appear that more than one strain of HIV can be isolated from a single individual [48]; this could result from infection by more than one strain, mutation *in vivo*, viral adaptation, or host cell selection from a heterogeneous virus population. Subjects who acquired a more neurotropic strain might be at risk for the earlier appearance of clinical neurologic disese.

Central to the issue of developing a model for HIV pathogenesis to the CNS is the characterization of the HIV isolates obtained from CSF and compared to corresponding isolates from blood. The degree of virus isolation from different cells and the cell tropism of the HIV viruses vary and is indicative of changes in the viruses. These findings are most likely due to *in vivo* changes [40,41]. The transmission of HIV may occur via monocytes before T lymphocytes become infected [42,43]. CNS isolates have been recovered which appear to be different in their biologic potential, and which are less cytopathic for monocyte/macrophages and replicate slower [42,44]. Virus isolates have become more virulent during a longitudinal isolation protocol [45] In addition, serological differences were found among CNS when compared to non-CNS isolates [46]. Characterization of HIV isolates from paired blood/CSF's collected in a longitudinal study may demonstrate changes in neurovirulence and the ability to replicate in CNS which would explain any lack of correspondence of CNS disease with systemic illness.

There is preliminary evidence that molecular differences in HIV strains are frequent and could explain biologic differences such as varying degrees of neurotropism. These molecular differences need to be examined in detail to gain an understanding of the nature of the isolates. Early studies showed that not only could Southern blots be used to detect HIV-1 gene sequences in various tissues from patients who had AIDS [47], but also that upon more detailed mapping using a variety of restriction enzymes, sequence heterogeneity was shown for HIV isolates [48]. Additional studies confirmed these findings and, restriction site sequence limited heterogeneity was found for autologous viruses isolated from each individual in comparison to isolates made from different individuals [49]. Genotypic variation was found among sequential isolates, but were more related to each other when compared to isolates recovered from different individuals [50]. Detailed sequencing analysis showed that there was greater heterogeneity (genetic polymorphism) among HIV isolates than had been previously suspected; however, the envelope gene region contained conserved domains. Other studies showed extensive variations in the envelope gene using restriction enzyme mapping [51]. This

is of interest because of the need to develop paradigms for HIV caused pathogenesis [52]. HIV biological and molecular variations were recently correlated. Six different envelope genes were isolated from an individual HIV isolate and were shown to exhibit different biologic properties in different cells [53].

Even though the goal of our grant is not to design experiments to elucidate the specific neurochemical mechanisms of HIV nervous system complications, we are anticipating working in this area or helping others. Our Human Neurospecimen Bank, storing the entire frozen collection of CSF/blood from the study correlated with precise psycho-neurological and immunological documentation, will provide a repository of fluid specimens for future basic science investigations by us and others.

(4) Work Accomplished (Tables are presented in Addendum 1).

GENERAL COMMENTS

The study population includes 137 individuals (as of Dec. 1, 1988) examined on all or part of the full battery of tests and procedures. This population can be separated into those with (n=102) and without (n=35) cerebrospinal fluid (CSF) examination. All participants have verbally agreed to have repeat CSF exams. Some participants have been lost to follow-up, 7 moved and are unreachable, 3 are bedridden, and 2 died. Of the 125 remaining in the longitudinal study, 81 have returned for one or more follow-up exams.

The data are presented in a series of tables, where each table presents data from one or more measurement domains (e.g. demographics, clinical, CSF and blood lab, neuropsychological, neuro-performance, computerized memory, psychological/mood, and evoked potentials). The data in a given table are grouped according to the major independent variables of interest: immunological disease stage [asymptomatic (systemic) seropositive (ASP), AIDS Related Complex (ARC), and AIDS] determined clinically by CDC criteria as modified by the NAIID AIDS Clinical Trials Group (ATCG) (see methods), or by T4 lymphocyte cell count groups with further subdivision by the presence or absence of clinically determined neurological dysfunction (Neuro POSitive or Neuro NEGative, respectively). Analysis of Variance was used to compare parametric data among group , while chi-square contingency analysis was used for non-parametric data. In some analyses where the differences between ARC and AIDS were not significant, the two were combined into an ARC/AIDS group.

DEMOGRAPHICS OF STUDY POPULATION

Table 1A presents the demographics of the total population with and without CSF exam, and for the 3 clinical immunological HIV subgroups: systemically asymptomatic seropositive (ASP), ARC and AIDS. The groups were well matched in age and education and predominantly male. They were also well matched in the frequency of occurrence of previous drug history, and previous neurological or psychological problems. Table 1B presents the corresponding demographics for only those patients who received a CSF exam at baseline. There are no statistically significant differences between this subset and the total population.

CLINICAL FINDINGS

Medical findings are reported in Table 2A. Karnofsky score, a global rating of physical well being (see Methods), and total number of ARC symptoms increased significantly with worsening of disease; the frequency of occurrence of Oral Hairy Leukoplakia and zoster (shingles) also increased.

Table 2B presents the clinical neurological findings separated into major sign and symptom domains. With the exception of cranial nerve findings, there is a highly significant progression of neurological dysfunction from ASP to ARC/AIDS. The global characterization of neurologically positive vs negative (see Methods for definition) shows that about 25% of ASP are positive, while about 71% of ARCs are positive.

The most common neurologic finding was peripheral neuropathy (15% of ASPs). This was almost always distal peripheral sensory neuropathy. Dementia or cognitive dysfunction was present in 14% of ASP subjects, and the majority of these were mild (Grade 1). These subjects were still capable of working and leading independent existences. Although they had definite cognitive dysfunction and neurologic abnormalities related to HIV, the use of the term

"dementia" as defined by the DSM-III [54], "A loss of intellectual abilities of sufficient severity to interfere with social or occupational functioning", would be more appropriately applied to grade 2 and 3, moderate and severe, respectively. No ASP individual presented with higher than grade 1. 6% of ASP had motor findings. Individuals with ARC had essentially the same signs and symptoms as ASP, but frequency and severity was signifiantly increased. Dementia/cognitive dysfunction was 14% for ASP and 56% for ARC, CNS motor findings were 6% versus 39%, respectively, and peripheral neuropathy was 15% versus 56%.

CSF AND BLOOD FINDINGS

Table 3A presents the frequencies of occurrence of abnormal CSF and blood laboratory findings in the clinically defined groups ASP, ARC and AIDS. In general there were no significant differences between ASP and ARC in the frequencies of abnormal CSF findings. The AIDS groups was small, but there was a trend evident in decreasing frequencies for most measures. I-BBB IgG Synthesis rate by the Tourtellotte formula [75] was abnormal in 73% of ASPs and 79% of ARCs. Abnormal IgG Synthesis by the Link Index (IgG Index) showed almost identical results. The frequency of unique CSF IgG bands was about 58-60% in these groups, with similar frequencies for bands more intense in the CSF than in the serum. When evidence for abnormal I-BBB IgG synthesis was combined from the rate formula and bands, the percentage of abnormal CSFs went up to about 90% for ASPs and ARCs. In contrast to the very high percentage of abnormal IgG findings in the CSF, only 11% of ASPs and 32% of ARCs showed direct evidence for presence of HIV in the CNS by culture. 33% of the ASPs and 41% of the ARCs showed p24 antigen (≥ 10 pg/ml) in the CSF.

Blood findings tended to support the progression of HIV clinical disease from ASP to ARC, but also indicated that about half of the ASPs were relatively well advanced (but pre-ARC). Positive gp41 antibody occurred in all patients as expected. Likewise, very high positive frequencies occurred for all groups for p51, p55, p61, p70 and gp120 antibodies. The frequency of positive HIV cultures and p24 antigen (≥20 pg/ml) increased from ASP to ARC, while positive p24 antibody decreased. There was also a decrease from ASP to ARC to AIDS for positive p17 antibody. Abnormal T4 cell counts (< 430) occurred in 46% of ASPs and 76% of ARCs. The percentage of abnormal serum IgG bands was about 50% for both ASP and ARC.

Table 3B shows quantitative values for the various CSF findings. In concordance with the frequency data in Table 3A, there were no significant differences between ASP and ARC groups for any of the CSF measures, although there was a trend for more abnormal values in the ARC group than ASP.

Tables 3C and 3D show separation of the laboratory data by Neuro Positive and Neuro Negative subgroups (see Methods for definition). The only significant differences for CSF findings was higher I-BBB IgG synthesis rate in the Neuro Pos group, although every measure showed higher abnormal mean values for the Neuro Pos group. This is in contrast to the blood findings which were consistently significantly more abnormal in the Neuro Pos vs. Neuro Neg group, except for abnormal serum IgG bands..

Table 3E shows the lab data for subgroups based on the presence or absence of CNS motor signs (see Methods). As with the more global neurological dysfunction measure shown in Tables 3C & D, there was a trend for both CSF and blood measures to be more abnormal in individuals with motor signs. However, statistically significant differences were found only for CSF white blood cell count and CSF p24 antigen levels. A similar pattern of results occurred for subgrouping based on the presence of dementia or absence of dementia/cognitive dysfunction. A separate analysis (not shown) for only moderate to severely demented individuals verus none or mild dementia showed a statistically significant difference in CSF p24 antigen levels ($p<0.003$), with the most demented subgroup showing the highest levels.

NEUROPSYCHOLOGICAL (NPE) FINDINGS

Table 4A shows a comparison of Asymptomatic Seropositive (ASP) versus ARC patient subgroups for all NPE measures. The two groups did not differ significantly on age or education level. Five tests show significantly worse performance in the ARC group as compared to ASP: Digit-symbol substitution, Benton Visual Retention test both number correct and number of errors, and the grooved pegboard tests for both dominant and non-

dominant hands. However, 12 out of 13 tests show better mean performance for the ASP group over the ARC group.

Table 4B shows the comparison for all NPE measures of patients rated clinically as neurologically positive or negative. The two groups differ significantly on age but the education level was the same. Ten out of 13 NPE tests showed statistically significant worse scores for the Neuro Positive group than for the Neuro Negative group: Verbal Fluency, Rey Auditory-Verbal Learning for both average number of correct in the 5 learning trials as well as number recalled after a delay, Trails A and B, Block Design, Digit-Symbol Substitution, Benton Visual Retention Test (# correct and errors), and grooved pegboard (dominant and non-dominant hand). A comparison of Table 4 A and B shows that the subsample represented by clinically defined neurological disease is more impaired on the neuropsychological tests than the the ASP group as a whole. Subgrouping ASP and ARC into NeuroPos and NeuroNeg revealed no significant interactions (2 way ANOVA).

Table 4 C shows a comparison of ASP and ARC patients classifed by normal (>=430) versus abnormal (<430) blood T4 cell counts. 5 out of 13 NPE tests showed a significant T4 group effect: Trials A, Digit-Symbol Substitution, Benton (# errors), and grooved pegboard (dom and nondom). However, there was not always a consistent progression of worse scores from high to low T4 cell groups

COMPUTERIZED MEMORY (CME)

Table 5A shows a comparison ASP versus ARC patient groups utilizing computerized memory evaluations. Five tests show significantly worse performance in the ARC group : Name-Face Association for the 14 item test trials 2 and 3, reaction times in the divided attention task for both trials 1 and 2, and number of names recalled out of 6 on the last trial in the first/last names test. However, 17 of the 24 tests showed better mean performance for the ASP group over the ARC group.

Table 5B shows the comparison for all CME measures of patients rated clinically as neurologically positive or negative. The two groups did not differ significantly on education level. However, the neuropositive patients were significantly older (44 vs 33.4 years). Since the normative data for CME do not show any significant age-related differences in the range 30-45 years, we did not consider the age difference to be meaningful for the test results. Ten of 24 test scores were significantly worse for the Neuro Positive group: Trials 2 & 3, and the 30 min delayed recall of the 14 Name-Face Association test, reaction time in the divided attention task for both trials 1 & 2, trials 2-5 of the first/last names test, and the related delayed incidental memory recall score from the first/last names test. 21 out of 24 showed lower mean scores for the Neuro Positive group as compared to the Neuro Negative group.

A comparison was made of ASP and ARC patients separated by normal (>=430) versus abnormal (<430) blood T4 cell counts. The results were that none out of 24 CME tests showed significantly worse performance for the abnormal T4 group than for the group with T4 cells within normal limits. However, the general trend was lower (worse) mean scores for the abnormal T4 cell group than for the normal T4 cell count group. Subgrouping ASP and ARC into neuropositive and negative revealed no significant differences by 2 way ANOVA.

Table 5C shows the frequency of abnormal findings for the neuropsychological and computerized memory exams for ASP versus ARC/AIDS combined into one group. For both exams an abnormal finding was defined as the presence of 2 tests with scores less than 2 standard deviations from the standard norms for that test. For the neuropsychological battery only 1 ASP and only 8 ARC/AIDS (27%) showed abnormal test findings. The yield was higher for the computerized memory exam with 24% of the ASP and 52% of the ARC showing abnormal findings. The latter figures accord well with the results of the clinical neurological diagnoses.

NEURO-PERFORMANCE TESTING

Table 6A shows a comparison of ASP versus ARC and AIDS patient groups for upper extremity tests. Twelve tests of 23 showed significantly worse performance in the ARC and AIDS groups as compared to ASP: Hand tapping speed (non-dom), reaction time for both simple and two-choice procedures and for both dominant and non-dominant hands, large peg rotation, purdue pegboard (dom and nondom), sustension tremor (dom and nondom),

and random tracking (dom and non-dom). In general strength, resting tremor and vibration threshold were not sensitive to disease progression.

Table 6B shows comparisons between Neuro Pos and Neuro Neg subgroups for the upper extremity tests; the NeuroPos groups is significantly older. Eleven tests showed significantly worse performance for the Neuro Pos group, most of which overlapped with the tests sensitive to classification shown in Table 6A.

Table 6 C shows comparison between T4 cell count groupings for the upper extremity tests; the lower T-cell group is significantly older. Only 5 of the tests showed significant effects by T4 cell groups: reaction time and purdue pegboard (dom and nondom).

Table 7A shows a comparison of ASP versus ARC and AIDS patient groups for lower extremity tests. Six tests of 15 showed significantly worse performance in the ARC and AIDS groups as compared to ASP: foot tapping speed (nondom), simple reaction time (dom and nondom), and standing balance (eyes open and closed).

Table 7B shows comparisons between Neuro Pos and Neuro Neg subgroups for the lower extremity tests. Thirteen tests out of the 15 showed significantly worse performance for the Neuro Pos group.

Table 7C shows that none of the lower extremity tests were sensitive to T4 cell count grouping

PSYCHOLOGICAL/MOOD TESTING

Tables 8A, B and C show the results for the Brief Symptom Inventory [55] index scale scores, Beck Depression Inventory [56] and the neuro-behavioral ratings of behavioral disturbances. Psychological and mood changes tested were significantly related to clinical immunologic disease progression and the presence of neurologic disease. On the other hand, the number of blood T4 cells did not reveal any significant effects except for somatization. Patients are more anxious and depressed as their immunologic and neurologic disease progresses, but it is not clear whether this represents a reaction to the progression or whether it is an inherent feature of an increasingly compromised nervous system.

EVOKED POTENTIAL TESTING

Tables 9 A and B show the results for pattern visual evoked potential component P100 latency and the N100 and P300 components of the auditory evoked potential to rare stimuli in the tone-pip target detection task. There are no significant differences in any of the EP components.

Given previous data that showed the PVEP P100 component sensitivity to demyelination along the optic pathways in MS and other disorders, it may be concluded that the HIV patients do not show any related pathology in the visual system, at least in the first two clinically defined phases, ASP and ARC.

The absence of prolonged latencies, or even any trend toward increasingly latency of P300 with either progressive immune system dysfunction or even with the presence of HIV-related neurologic disease suggests that the P300 component is not sensitive to neurocognitive dysfunction seen in HIV patients. This is in strong contrast to findings in Alzheimer disease and a broad range of other dementias, all of which show significantly prolonged P300 latencies in group analyses when compared to age-matched normal controls [57]. Of course it is possible that P300 is not sensitive to the rather early stage of neurologic dysfunction that the majority of our HIV patients appear to represent. However, even when we analyzed separately the small group of 8 moderately to severely demented HIV patients, only 2 (25%) had abnormally prolonged P300 latency.

This is in marked contrast to the results of behavioral testing (Tables 4-8), where many of the tests in the 4 domains of testing show sensitivity to early changes in cognitive function in the HIV patients.

MRI

Over 70 baseline MRI's of brain have been performed on study participants. All MRI's are performed according to a standardized protocol and include coronal and saggital sections, and T1 and T2 weighted imaging. All are interpreted immediately for clinical purposes by the neuroradiologist at the MRI facility and reviewed by the study neurologist. Additionally, the

films are reviewed by a second neuroradiologist who is "blinded" to the subject's diagnosis and fills out a standardized form describing any abnormalities.

32 of the 70 MRIs to date have been interpreted by a 'blinded' neuroradiologist: 17/32 were ASP and 15/32 were ARC. 6/32 ASP/ARCs had abnormal MRIs, 1 individual had cortical atrophy, 3 had discrete white matter lesions on T2 weighted imaging, 1 had a lesion compatible with old trauma; one had a large cisterna magna (congenital). Only one of these individuals had cognitive abnormalities and CNS motor abnormalities on neurologic exam. 26/32 ASP/ARCs had normal MRIs; of these 12 had cognitive abnormalities and CNS motor signs on clinical exam. To date, these results suggest that brain MRI abnormalities are not consistently associated with clinical neurologic status in HIV seropositive individuals who do not have CNS opportunistic infection or tumors; they are more helpful to "rule out" mass lesions in clinically abnormal individuals prior to CSF exam, and as a baseline to which future MRIs will be compared. This would concur with the findings in a previously published report [57a] which found that the clinical diagnosis of HIV encephalitis usually antedated the radiographic diagnosis, and that CT and MRI were relatively insensitive in detecting the primary changes of HIV encephalitis.

PRELIMINARY DATA (Florida Service Project)

Characteristics of the 19 patients with paired HIV blood and CSF isolates are shown in Table 10.

USE OF HUMAN NEURAL CELL LINES FOR HIV CHARACTERIZATION.

HIV-1 culture in human neuroblastoma cells. Early studies revealed that HIV_B did not propagate in African monkey kidney (Vero) cells, human cervix carcinoma cells (Hela), and human brain astrocytoma cells, as evidenced by the inability to detect reverse transcriptase (RT) activity in cell culture supernatants. However, HIV was able to propagate in neuroblastoma cell lines attaining peak RT activity 10-14 days post-infection. HIV was also detected by using the p24 antigen capture ELISA and by antibody inhibition of reverse transcriptase activity. After prolonged growth in cell cultures, there were additional elevations of released RT activity, 20-fold greater activity than the initial peak, lasting from 36 to 74 days and 110 to 140 days post-infection. These experiments were repeated nine times. The two neuroblastoma cell lines used are highly susceptible to HIV, are used for HIV-1 replication and isolation studies, and provide a model related to HIV persistence in CNS cells [58,59]..

Preliminary evidence for biological differences among HIV-1 isolates. *In situ* hybridization was used to detect HIV-1 in HIV-1 infected neuroblastoma cell cultures. *In situ* hybridization detection of patient isolates of HIV-1 in these cell lines peaked at 6 weeks post-infection. At six weeks, there was greater target detection in neuroblastoma cells infected with HIV-1 isolated from patient blood ($HIV\text{-}1_{PBL}$) than in neuroblastoma cells infected with HIV-1 isolated from patient CSF ($HIV\text{-}1_{CSF}$). In both cases, HIV was detected only inside cells although there was released HIV detected once, at 29 days, for the neuroblastoma cell culture infected with $HIV\text{-}1_{CSF}$. The target detection and % of $HIV\text{-}1_{CSF}$ infected cells decreased more rapidly 6 to 12 weeks post-infection than the % of $HIV\text{-}1_{PBL}$ infected cells. However, the input dose of HIV was not standardized and titrated.

***IN SITU* HYBRIDIZATION USING ^{3}H-HIV-1 cDNA Probe**

Cells	HIV-1 Background (grains/ nucleus or cell)	HIV-1 Signal (grains/ nucleus or cell)	% positive cells	RT activity in supernatants
Lymphocyte cell line				
HUT-78 (uninfected)	<0.2	0	0	negative
HIV-1_B infected	<0.2	20-30	>90%	positive
Neuroblastoma cell line				
SK-N-MC (uninfected)	<0.2	0	0	negative

PT-HIV-1_{PBL} infected	<10	30-40	<15%	negative
PT-HIV-1_{CSF} infected	<10	<30	<10%	positive at 29 days only

(Shapshak et al, unpublished results, 1988].
HIV-1 isolates were the laboratory strain (HIV-1_B) and clinical strains isolated from patient (PT) blood (HIV-1_{PBL}) and from CSF (HIV-1_{CSF}).
RT = reverse transcriptase. Assay according to Lee et al, [60,61].

TENTATIVE CONCLUSIONS

We have confirmed and extended our earlier publications, that were the first to report that I-BBB IgG Synthesis by rate and bands is abnormal at an early stage of HIV infection [16,17]. This supports the contention that HIV begins to exert its effects within the CNS early and prompts an I-BBB inflammatory response. We found that about 88% of the ASPs and about 91% of ARCs showed this evidence of inflammation in the CNS. It is our notion that these results are due to viral penetration of the CNS and represent de novo I-BBB IgG synthesis. The Tourtellotte equation, which corrects for passage of IgG across an abnormal leaky BBB, quantifies the rate of IgG synthesis and is reflective of the secretion and number of plasma cells inside the BBB. Additionally, the precise concordance of the IgG Index with our I-BBB IgG synthesis rate formula is also evidence of inflammation which contains plasma cells inside the BBB. Further, utilizing an entirely different method, the presence of unique CSF IgG oligoclonal bands not present in serum, also indicates the presence of plasma cells in the CNS.

In our study, mean I-BBB IgG synthesis (mg/day) increased from ASP through ARC and declined again in AIDS. This is consistent with previously published data [18,62]. Likewise, the IgG index was elevated in early-middle systemic disease and decreased in AIDS, again consistent with other reports [18,63]. The percent of subjects with elevated CSF WBC was high in ASP/ARC and diminished in AIDS, (although mean cell count was most elevated in the AIDS group, which was due to one outlier with a very high cell count in this small group), again consistent with previous reports [18]. The incidence of subjects with unique CSF IgG bands was highest in ASP and ARC and lower in AIDS. The mean number of bands did not change significantly across disease stage groups. Decrease of the frequency of oligoclonal banding with disease progression has been previously reported [21,64]; there is a far less marked change reported by Marshall [18]. HIV p24 antigen level in CSF was higher in the ARC and AIDS groups (12.1, 9.8 respectively) than in ASPs. Concentration of HIV p24 antigen in CSF was not found in any of the samples studied by Marshall [18] but this essentially agrees with previous findings that HIV p24 antigenemia is associated with clinical deterioration [20]. In blood, HIV culture, p24 antigen, T4 count, and T4/T8 ratio all followed the pattern of increasing systemic illness. Percent of subjects with serum antibody to p24 decreased slightly with disease progression (ASP vs ARC/AIDS), while antibodies to gp41, p51, p55, p61, p70 and gp120 remained stable. Of interest, antibodies to p17, another core protein, decreased significantly from ASP to AIDS. It has been proposed that decline of anti-p17 antibody is an even earlier marker for disease progression than anti-p24 [65]. Loss of p17 antibody is thought to be associated with increased p17 production and the formation of antigen-antibody complexes which are not measured in the antibody assay. Anti-p17 is also thought to have some neutralizing properties [66]. It would be of interest to see if there will be a concurrent decrease in the CSF of anti-p17, which will be investigated in the proposed study. In summary, the majority of our results as analyzed across groups of subjects in different levels of systemic disease are generally in line with the finding of other studies. Thus our population appears very representative of HIV infected individuals in the early and middle stages of HIV systemic disease.

Our CSF data was also analyzed between groups with HIV related neurologic disease and no HIV related neurologic disease and by type of neurologic disease. It is in this analysis that statistically significant information as well as new significant trends emerges. Unlike Marshall's study, our group contains large number of both neurologically normal and abnormal subjects both in early (ASP) and middle (ARC) disease. There was a very low incidence of CNS abnormalities in subjects from Marshall's study, which may reflect both the very large numbers with very early illness, their youth (mean age in his study was approximately 10 years lower than ours -personal communication, Marshall, Nov. 1988), and their selection by military recruitment personnel for overall good physical health. The low

incidence of neurologic disease may also be explained by a presumed lower rate of drug and alcohol abuse in the Air Force sample(these factors were not listed in his study), or by a presumed lower incidence of untreated exposures to infectious diseases that could act as cofactors for emergence of neurologic disease (i.e., syphilis, hepatitis B, CMV).

While a generally low rate of neurologic disease in relatively healthy, early HIV disease stage individuals may be a correct epidemiologic representation of Marshall et al's population, it does not give them a sufficient number of abnormals at a similar stage of disease to compare CSF values to. It is one of our goals to determine which values, if any, act as a distinguishing marker for neurologic abnormality.

When CSF values were analyzed by the presence of any HIV related neurologic disease (neuro POS) versus no HIV related neurologic disease (neuro NEG), I-BBB IgG synthesis rate was significantly ($p < 0.003$) more elevated in neuropositives, as were blood values for p24 antigen ($p \leq 0.03$); absolute T4 count ($p < 0.004$), and T4/T8 ratio ($p < 0.004$) were significantly lower. There was a trend ($p = 0.16$) for elevated CSF p24 to be related to neuro POS. This information confirms the overall association of neurologic disease with deteriorating immune function as measured by blood parameters. Further, the finding of a more intense inflammatory reaction in neuropositives as measured by intra-BBB IgG synthesis rate, is similar to findings reported by Elovaara [63] and McArthur et al [67]. While p24 in CSF was not significantly associated with neurodisease, all important systemic immune parameters evaluated to date (Serum p24, T4, and T4/T8 ratio) were highly significantly associated with the presence of HIV related neurodisease.

When neurologic disease was broken down into subcategories, the presence of clinical cognitive abnormalities (dementia, grade 1-3) was associated with a trend toward higher ($p \leq 0.08$) levels of intra-BBB IgG synthesis rate and CSF p24 antigen ($p = 0.08$), and significantly associated with positive HIV culture ($p \leq 0.04$), and lowered T4/T8 ratio ($p = 0.05$). If a subgroup of our most demented patients (grade 2 & 3) were analyzed, HIV p24 CSF levels became statistically significant ($p < 0.05$). When our group was broken down into those with and without CNS motor signs, CSF cell count ($p \leq 0.003$) and HIV p24 in CSF ($p \leq 0.01$) were significantly higher in those with CNS motor signs. CNS motor signs usually appear after cognitive abnormalities and this group overlaps with those who have moderate to severe dementia.

The subset of patients, defined by the presence of HIV related clinical neuro disease as evaluated by the neurologist, showed pronounced declines on most of the performance tests in all 3 domains of performance testing (computerized memory, neuro-psychologic, neuro-performance), as well as in psychological/self ratings. A pattern of performance deficits emerged from our standardized Human Performance Laboratory. Finger dexterity, reaction time, hand/arm coordination, and one-legged standing balance were the most sensitive indicators of HIV effects on the CNS. Of great importance is that these objective measurements confirmed in the majority of the cases historical and exam information of disability elicted by the neurologist. However, there are also pervasive effects of HIV disease progression on mood and other psychological variables. A key issue is the direction of effects on these measurement areas. Does HIV-related CNS disease affect the neuro systems underlying both mood and behavioral disturbances on the one hand, and cognitive/motor deficits on the other? Or are the primary effects on the mood system, with secondary effects on neurocognitive systems? Certainly, the progression of HIV effects to a full-blown dementia argue for the former case. A more complete view of the relative longitudinal changes in the various systems may help to clarify the situation at earlier stages of the disease.

Data presented in this report are a cross sectional analysis of baseline evaluations conducted on an HIV seropositive sample of 137 individuals. Data presented showed evidence of clinical HIV related neurologic disease in 25% of ASP, 71% ARC, 100% AIDS. We do not contend that this is an accurate representation of the incidence of neurologic dysfunction, but believe that by following a sufficient number of asymptomatic seropositives with no evidence of neurologic dysfunction for 3 to 5 years we should arrive at a more accurate estimate of the incidence and potential risk factors of neurologic dysfunction. The fact that we do have a wide spectrum of disease in our cohort lends an advantage in that we can perform a cross sectional and longitudinal analysis of possible serum and CSF markers for HIV neurologic disease. While

no marker has been found to date, we have confirmed our original observation that HIV penetrates the CNS early. Additionally, we have quantified differences in CSF constituents between subjects with and without HIV related neurologic disease.

We believe that the design and study population of our study is relatively unique and valuable. Unlike Marshall's population, our group is probably more representative (in terms of age plus risk factors) of the current American population with HIV. In that sense it complements the ongoing Air Force study. The MACs study is another longitudinal study of HIV infected men. Unlike our study, it is an epidemiologic study of the overall natural history of AIDS. Its' primary focus is not on the nervous system, but on the overall development of symptoms and their relationship to high risk behaviors. The MACS have a neuropsychologic subproject in which all subjects get a neurologic questionnaire and take a battery of computerized neuropsychologic tests. Only subjects in the MACS with abnormal screening tests (who can be seropositive or negative) are selected to receive a full neuropsychologic and neurologic exam. Subjects are requested, but not required, to donate CSF. CSFs are currently analyzed only for cell count, total protein, glucose, VDRL, and intra-BBB IgG synthesis (rate and bands). MRI's are not performed on all participants, and p24 antigen and other possible markers are not evaluated. In addition to pursuing an advanced basic science approach to I-BBB events and CSF markers for disease, our study allows us to explore the mechanisms for HIV neurologic disease (which is not a goal of the MACS). We feel that MACS may be missing subjects with clinical neurologic disease that is missed by their neuropsychologic screening battery.

Even though the penetration of the CNS is early, the behavioral and neurological signs and symptoms remain subclinical in the majority of cases for an undetermined time. In addition, we are still lacking clear-cut indicators or predictors of the temporal onset and severity of behavioral consequences of early HIV penetration of the CNS. It is our contention that our longituidinal design will help to elucidate the duration of this subclinical period as well as mechanisms and markers for clinical deterioration.

(5) CURRENT METHODS.

The purpose of this study is to correlate CSF parameters with clinical and laboratory measures of HIV disease progression using a longitudinal design. The key issue is to determine what markers exist for the appearance and progression of neurologic disease in CNS HIV infection.

The original study design, submitted in May, 1986, called for 50 ARC and 50 AIDS subjects, with and without neurologic disease; and for 30 MS patients and 50 "normal" controls (from the National Neurological Research Bank, NNRB). It became apparent that this design required modifications for the following reasons:

1. A very high proportion of AIDS/ARC subjects that were examined had active, ongoing clinical neurologic disease. This suggested that intra-BBB changes and possibly subtle neuropsychological abnormalities which preceded clinical disease were being missed.
2. Evidence accumulated through the efforts of this laboratory, and others, that the CNS was infected very early in the course of HIV disease; and that signs of CNS inflammation (such as elevated I-BBB IgG synthesis) are present in many systemically and neurologically asymptomatic persons. Additionally, investigators such as Grant et al [9] proposed that cognitive deficits were present in asymptomatic seropositive individuals.
3. Increased information was gained about the latency period from acute infection to ARC and AIDS suggesting that it may take up to eight years or longer for individuals to become clinically ill.
4. Follow-up examinations of many of the ARC/AIDS patients is impossible because they become bedridden, move out of the area or home to be cared for by their families, or die.

The design of this study has been therefore modified as follows. We will continue to follow as many of the AIDS/ARC patients recruited as long as possible, and expect that a substantial number of the ARC patients will advance to AIDS. We have recruited 67 asymptomatic seropositive subjects as of Dec.1, and an additional 30 since Dec. 1 for a total ASP group of 97. We will attempt to recruit approximately 33 ASPs more, with a continuing emphasis on subjects whose absolute T_4 counts are above 500. The goal is to have 120 ASP

patients enrolled in the project so that about 100 can be followed for at least 3 years. This would take into account an attrition rate of about 20%. In addition we will continue to follow the approximately 70 ARC/AIDS who have already been recruited.

We have also begun to test high-risk HIV seronegative controls. These volunteers have primarily been the seronegative spouses, friends and cohabitants of our infected subjects. It is our goal to recruit at least 30 high risk seronegative control subjects age-matched with the group of HIV seropositives. These controls will provide group-specific base rates for laboratory and clinical abnormalities. It has become clear that the normal control and neurological disease control fluids banked in the NNRB are adequate to serve as control fluids for only some CSF and serum parameters. It is necessary to determine the base rates of clinical and behavioral lab parameters for a matched high risk seronegative population. Also this increases the opportunity to investigate events which occur at or near sero-conversion. Although the study is not designed to focus on such events, their appearance will afford pilot data for future studies.

An important aspect of this project is the development of a multifactorial attack based on focused clinical and laboratory examinations and analyses. There is a core unit which admits the subjects and follows up. Part of this core unit is the basic medical examination and clinical assessments. The standardized neuropsychiatric questionnaire/examinations, neuropsychological test battery tests, neuroperformance tests, and computerized memory evaluations are another major component. The CSF/blood examinations are then conducted and the fluids are stored in the HIV section of the Human Neurospecimen Bank for later analyses by the study investigators and potentially by other investigators. In addition, through the neuropathologists associated with the Bank, any post-mortem brain tissue that becomes available from study participants may be stored cryopreserved by the Bank and characterized by these neuropathologists.

Subjects were recruited into an ongoing longitudinal study of the effects of HIV on the nervous system, conducted by the Neurology Service, VA Medical Center, West Los Angeles, Wadsworth Division. All subjects signed an IRB approved consent form. Sources of subjects included newspaper advertisements, referrals from private physicians, and increasingly, referrals from other subjects. Our sample was largely white, male, homosexual, and well-educated (see table 1A). The current population consists of 67 ASP, 60 ARC, 10 AIDS (shown in Table 1A), plus an additional 30 ASPs recruited since Dec. 1, and 8 seronegative high risk controls. We will recruit an additional 33 HIV seropositive individuals who meet the criteria for the systemically asymptomatic subgroup, as well as at least 22 additional HIV seronegative high risk controls.

Subjects were administered standardized questionnaires covering their medical and neuropsychiatric history, a standardized physical and neurologic exam, neuropsychologic testing, computerized memory and neuro-performance testing. Blood was drawn for T cell subsets, viral cultures, HIV antibody levels, and p24 antigen levels. Lumbar punctures were performed in a standardized fashion and CSF samples were immediately placed on ice. Most of the subjects also underwent one or more of the following: Magnetic Resonance imaging (MRI) of the brain and evoked potentials. Individuals whose initial evaluation demonstrated an active, ongoing CNS secondary infection or CNS tumor were excluded from the study population. Some subjects were also studied as part of our laboratory's participation in a neuropsychologic study of persons enrolled in the VA Cooperative Study 298 (a double blind, randomized clinical trial of AZT versus placebo for persons with ARC) who received pre and post treatment LP's. Some of these subjects did not receive our full work up, but the 298 investigators have kindly opened their records to us and many of their evaluations overlap with ours. Additionally, this laboratory participated in ATEU 005, an NAIID sponsored double blind, placebo-controlled randomized multicenter trial of AZT for AIDS-Dementia. The subjects in this study received this laboratory's fully designed work up, including serial LP's and neuropsychologic testing after treatment according to the ATEU 005 protocol.

All subjects were classified by the techniques established by the principal investigator and his trained staff of neurologists as follows: Systemically, by an infectious disease questionnaire, physical exam, and determination of T cell subsets; neurologically, by a standardized neuropsychiatric questionnaire and exam. Skin testing was not obtained because

of the difficulty with compliance, the need for extra visits by working individuals, and because of our initial lack of personnel during the first 12 to 18 months. We feel this is not a serious deficit, as some investigators have found it difficult to classify all subjects by the Walter Reed System and feel that the absolute T4 count, perhaps in conjunction with beta-2-microglobulin levels, is still the best indicator for progression. In one study, only 133 of 431 homosexual men followed in a longitudinal fashion could be assigned a Walter Reed Stage, and anergy was useful only in Walter Reed 5 as a predictor of AIDS [68].

Subjects were classified as follows :

(1) **Asymptomatic seronegatives at high risk seroconversion(ASNHR)**
(2) **Asymptomatic seropositive subjects (ASP)**
(3) **AIDS-related complex (ARC)**
(4) **AIDS**

Asymptomatic high risk seronegatives (ASHRSN) were healthy persons with negative HIV antibody tests, viral cultures, and Western Blot who had engaged in activities likely to infect them with HIV (e.g. sexual activity with an HIV positive partner). These subjects correspond to Walter Reed Stage 0.

Asymptomatic seropositive subjects (ASP) were positive for HIV antibody with or without lymphadenopathy who had no opportunistic infections or other systemic manifestations of HIV. These subjects also had a Karnofsky score of 100% if nervous system symptoms were excluded. This category corresponds roughly to CDC II and III, Walter Reed stages 1-4 and the entry criteria of ATEU 019 [130](a double blind, randomized placebo-controlled clinical trial of AZT for ASP's). T cell subsets could be normal or abnormal in this group, but usually were over 200.

AIDS-related complex (ARC) was defined by the presence of one or more of the following:

1. Either by physical exam or history: Oral candida (thrush) documented by morphology or by response to antifungal therapy in the past 3 years.
2. Oral hairy leukoplakia.
3. Shingles (herpes zoster) in the past three years.
4. Chronic intermittent diarrhea of at least one month duration with at least 3 liquid stools/day, worked up without a definable cause.
5. Unintentional weight loss of at least 10 pounds or 10% body weight, worked up without a definable cause in the past 3 years.
6. Drenching whole body night sweats (on at least 3 occasions in the previous 3 months).
7. Recorded temperatures of at least 100°F for at least one month.
8. Chronic fatigue which interfered with normal activity at least 1-2 times per week for the past 6 months.
9. Recurrent seborrheic dermatitis or tropical pruritic folliculitis, whose onset occurred within the estimated time of infection.

There were tests taken from both inclusion criteria for ATEU 016 [131] and exclusion criteria from 019 [130].

AIDS was defined as follows: individuals who had at least one medically documented episode of pneumocystis cariini pneumonia (PCP), extraintestinal strongyloidosis cryptococcal meningitis, toxoplasmosis, cryptosporidiosis, isoporiasis, esophageal or bronchial candidiasis, mycobacterium avium intracellulare or M. Kansaii infection, progressive multifocal leukencephalopathy (PML), chronic mucocutaeneous herpes simplex (HSV) or disseminated herpes simplex, cytomegalovirus infection (CMV), or other infections listed by the CDC [132,133]as AIDS-defining; or who had biopsy-documented Kaposi's Sarcoma (KS), or other syndromes such as chronic lymphoid interstitial pneumonitis. These subjects correspond roughly with WR6 (except for K.S.) and CDC IV C-1,IVD, and IVE. Subjects with the so called AIDS-Dementia complex who have no other systemic manifestations of AIDS are not included by us in the "AIDS" group.

The criteria we used are taken from the AIDS Treatment/Evaluation Unit (ATEU) (now called AIDS Clinical Trials Group, or ACTG) entry criteria for their major multicenter studies of the treatment of asymptomatic seropositive, ARC, and AIDS. Our rationale for using the ATEU entry criteria rather than the CDC was as follows: Initially we planned to have a strong

affiliation with the UCLA ATEU and to recruit the majority of our subjects from them. This would have allowed us to save money on clinical laboratory and to recruit previously worked up patients. As subjects were entered into double blind treatment trials, the effects of AZT on the CNS would have been studied simultaneously. Thus we planned to use their classification system, which is a modification of the CDC and differs primarily in that subjects with generalized lymphadenopathy by CDC criteria have been considered "asymptomatic", and that some constitutional and dermatologic symptoms have been added to the CDC criteria for ARC. Further, the ATEU criteria did not include any neurologic symptoms as AIDS or ARC defining; subjects with AIDS-Dementia were considered separately in another study (005). It so happened that our most successful recruitment sources have been newspaper advertisements and word of mouth rather than the UCLA ATEU. We were also able to get more previously untreated, drug free subjects to follow in this manner. We continued to use ATEU criteria to group our subjects as ASP, ARC, AIDS and simply began to simultaneously perform the CDC staging on each subject.

We classified neurologic symptoms separately from systemic disease, as the relationship of HIV-related neuropathology to the other aspects of the illness are not clear and have never been defined in a clinical study. Subjects were categorized as follows: Neuro-negative (no HIV-related neurologic disease) or neuro-positive (HIV-related neurologic disease). The presence or absence of neurologic disease unrelated to HIV was noted in both of these two primary categories. Unrelated neurologic disease was defined as neurologic disease that predated HIV exposure or had another, clear cut cause. In our sample this generally consisted of chronic tension or migraine headaches, chronic musculoskeletal low back pain, cervical or lumbar disc disease, or post traumatic neuropathy. This category also included confounding problems such as severe head injury with loss of consciousness, alcoholic dementia or schizophrenia and subjects with the latter problems were excluded from the neurologic and behavioral analysis. HIV-related neurologic disease was subclassified as cognitive (dementia), CNS motor (weakness, spasticity; movement disorder), peripheral neuropathy and other. Behavioral abnoramlities such as depression, emotional lability, and changes in social behavior were noted and graded but were not considered to make a subject "neuropositive" as in most cases it was impossible to toss out previous psychiatric problems or reactive depression from primary behavioral changes caused by the virus. Subjects needed to have complaints and/or signs consistent with a neurologic syndrome to be diagnosed as neuropositive (for example, subjects with isolated slowing of eye movements were noted but not called "neuropositive"). These syndromes were further classified as "absent, (0), mild (1), moderate (2), or severe (3), according to a grading system taken from the ATEU 005 protocol [69] (see addenda).

The grading system used for neurologic problems (i.e., dementia, CNS motor, peripheral neuropathy) and behavioral problems we used was adapted from the ATEU 005 [69] protocol which divided its summary assessments into 0 - normal, 1-mild, 2-moderate, and 3-severe.

Dementia

1 mild dementia (symptoms or mild signs, but not functional impairment
2 moderate dementia (functionally impaired so that cannot do work or demanding tasks, but can simply interact socially
3 severe dementia with very limited intellectual or social interaction.

Overall CNS motor

0 none"
1 mild symptoms or signs but independent ambulation and ADL
2 moderate symptoms and signs, needs prompts or assistance with walking or ADL
3 Severe symptoms and signs, nonambulatory or can walk few feet with full assistance.

Overall peripheral neuropathy

0 none
1 mild weakness or sensory loss
2 moderate, needs assistance to ambulate or with hand tasks
3 severe, unable to ambulate or do handtasks

Overall behavior disturbance:

0 none
1 Mild symptoms or signs but able to function socially normally or close to normally.

2 moderate symptoms and signs - interferes with normal social interaction or capacity to work
3 severe symptoms and signs requring nearly constant attention or institutionalization.

In our opinion, grade 1 Dementia does not meet the DSM-III definition of dementia which is "loss of intellectual abilities of sufficient severity to interfer with social or occupational functioning" [54] and only grade 2 and 3 are properly termed dementia while 1 might be more approxiately called "cognitive dysfunction".

In addition to the above criteria, the presence or absence of chronic, recurrent oral or genital herpes simplex infections, syphilis, viral hepatitis, and previously diagnosed psychiatric illnesses such as depression, the use of psychotropic medications and substance abuse were also recorded but not used to classify subjects. Demographic information such as employment, age, sex, handedness, education, race, and risk factor were also recorded.

Neuropsychological Tests. The Neuropsychological test battery consisted of the following tests: a 10 yd timed gait test (TG), Finger Tapping with dominant (FTD) and nondominant (FTN) hands, Verbal Fluency (VF), Trailmaking A (TrailsA), Trailmaking B (TrailsB), Digit Symbol Substitution (DS), Vocabulary (Vocab), REY Auditory Verbal Learning Test (REY), Block Design (BD), Digits Forward (DF) and Digits Backwards (DB), Grooved Pegboard with dominant (GPD) and nondominant hands (GPN), and the Benton Visual Retention Test (BVRT). These tests were adapted from the ATEU 005 protocol as those considered most sensitive to HIV related neurological disease. The results to date support the sensitivity of these tests (see Work Accomplished). We will utilize standard norms for each test, in addition to data from the seronegative controls, to assess abnormal performance on these tests.

Computerized Memory Tests. The Computerized Memory Assessment system is composed of a 19" color video monitor with a coordinate touch screen, a laser disk player, and a personal computer. The system is leased from Memory Assessment Clinic, Inc. of Bethesda, MD. A battery of six tests was administered to the subjects: Name-Face Association (NFA), Misplaced Objects (MO), Divided Attention (DA), First-Last Names (FLN), Telephone Dialing with Interference (TDI), and Incidental Memory (IM). These tests were selected to reflect the everyday memory problems that HIV patients often complain of.. The results to date support the sensitivity of these tests (see Work Accomplished). Extensive norms are available from the Memory Assessment Clinic, Inc., and we will use data from the seronegative controls to assess abnormal performance on these tests.

Neuro-Performance Tests. The Neuro-performance evaluation (NP) consists of 20 measures designed to assess a subject's coordination, simple reaction times, multiple reaction times, vibration sensitivity, hand and foot speed, resting and sustention tremor, two dimensional tracking abilities, manual dexterity, strength, and balance. The evaluation was performed using a computerized performance battery developed by George Kondraske and his associates at the University of Texas at Arlington, Center for Biomedical Engineering, and other standardized instruments. The upper and lower extremities were tested separately. The results to date support the sensitivity of these tests (see Work Accomplished). Extensive norms are available from the Dr. Kondraske's group, and we will use data from the seronegative controls to assess abnormal performance on these tests.

SERUM AND CSF SAMPLES Matched blood and CSF samples were collected, cell counts performed, cells deposited on glass slides for ISH and PCR, and the remaining cells aliquoted and stored at -70°C as previously described [73,74,75]. IgG, albumin, and oligoclonal IgG banding were determined as documented [70,71, 73,74,75]. Matched blood and CSFs from normal controls and other neurological diseases, which were utilized as controls, were obtained from the Human Neurospecimen Bank [72], Neurology Service, Wadsworth VAMC.

INTRA-BBB IgG SYNTHESIS RATE FORMULA. IgG and albumin (Alb) concentrations were quantified by electroimmunodiffusion (EID) and the intra-BBB IgG synthesis rate (milligrams per day) was calculated according to the equation [73,74,75]. I-BBB IgG Synthesis rate (mg/day)=

$$\left(\left(IgG_{CSF}-\frac{IgG_{Serum}}{369}\right)-\left(Alb_{CSF}-\frac{Alb_{Serum}}{230}\right)x\left(\frac{IgG_{Serum}}{Alb_{Serum}}\right)(.43)\right)x5$$

where concentrations are in milligrams per deciliter, 369 and 230 are the average normal serum/CSF ratios for IgG and albumin, respectively, 0.43 is the molecular weight ratio of albumin/IgG, and 5 is the daily CSF production in deciliters.

Leakage of albumin through the BBB was determined by the albumin leakage formula:

$$\{Alb_{CSF}-[Alb_{SERUM}/230]\}x5$$

OLIGOCLONAL IgG BAND DETERMINATION. Oligoclonal IgG bands were detected by isoelectric focusing electrophoresis (IEF), IgG-specific immunofixation and silver nitrate staining as described [70,71]. Oligoclonal IgG bands are defined as IgG bands found exclusively or more intensely in CSF compared to autologous serum.

POLYMERASE CHAIN REACTION (PCR). PCR will be carried out using appropriate primers chosen carefully for viral detection. The PCR experimental design includes use of DNA and RNA from cells from the buffy coats and CSF and from both cryopreserved and paraffin embedded CNS tissues . Analysis of the amplified products is to be done using Southern and dot blots. Short synthetic probes and prepared and end-labeled for detection. Multiple primer pairs are used in order to avoid false negatives due to viral mutations. PCR is done with these primers in control tissues, viral infected cells, uninfected cells, and in cells infected with other viruses. This constitutes an important control sequence for PCR. Additional control experiments for the PCR procedure are especially important for each new set of primers and probes are the following: 1) serial dilutions of measured quantities of cloned target sequences in carrier DNA, preferably from similar preparations, will be amplified and detected to establish absolute limits of sensitivity; 2) selection of proper target sequences in the viral genome. A careful selection must be made by inspection, taking into account the following additional aspects of target sequences: availability of target sequence information; avoidance of human homology regions; avoidance of sequence variability in the primer and probe regions; absence of intrastrand complementarity; and presence of proper G+C content.

In situ HYBRIDIZATION AND TISSUE STAINING IN CRYOSECTIONS AND FORMALIN FIXED SECTIONS. The *in situ* hybridization technique successfully and originally employed by Brahic and Haase utilized measles virus and Visna virus cDNA [76,77,78] and frozen thin sections of brain. We have applied modifications of this technique to our studies with increased stringency [79,80-89] We will apply specific HIV probes from the 6-5 region of the genome to cells collected from buffy coats, cells from CSF as well as post mortem tissue. For the tissue cryostat 10u sections and 4u sections of FFPE tissue are deposited on coated slides and treated with proteinase K. The hybridization reaction utilizes 2 nanograms or less of ^{35}S, ^{3}H-, or ^{125}I-DNA or RNA in 5 microliters/cm^2 of tissue for each slide. The hybridization solution contains 50% formamide, salts, and buffer, and the reaction is carried out under siliconized cover slips at room temperature. Although we currently use the published procedure, we explore the stringency of the hybridization reaction for each probe by varying temperature, salt, and formamide concentration during hybridization and the washes. After extensive washing, the slides are treated with Kodak NTB-3 nuclear track emulsion, using the dipping method. After processing, autoradiography is performed and the slides are developed. The *in situ* hybridization procedure generally results in reduced crispness of the histology. Therefore, we always cut multiple step sections and stain several sections without ISH. Routine Giemsa and Toluidine Blue staining methods are used to gain definition of the cells within which the hybridization occurred. In addition, we stain with other classical stains such as Hematoxylin-Eosin, Oil Red O, and Cresyl Violet. We use both light and dark-field illumination with the new Olympus-McBain automated microscope just purchased to enhance cellular definition and grain appearance.

TEST OF IMMUNOLOGICAL FUNCTIONING

These tests (absolute number and percentage of lymphocytes, T4, T8 subsets in absolute number and percentage, T4:T8 ratio) were performed at Professor J. Fahey's laboratory in the UCLA School of Medicine according to Fahey et al, [90].

MATERIALS AND METHODS FOR SERVICE PROJECT

1. Subjects. HIV virologic studies are performed on subjects enrolled in the study and chosen by the Principal, Investigator.
2. Specimen coding by the Principal Investigator and transmittal from Los Angeles to Miami Beach. Of major importance, the cooperation among the two groups has led to an excellent working relationship. This study has been ongoing for nearly three years and there has been no incident whereby samples have been lost, mislabeled, contaminated, or spilled. Specimens are shipped, next day delivery, from the Human Neurospecimen Bank, at the Neurology Service, Wadsworth Hospital Veterans Administration. Neither names nor other personal identifiers are given and the accurate, efficient, and anonymous consecutive number system of the Bank is maintained in these studies.
3. HIV isolation from blood and CSF. These procedures are performed as previously described [16,17].
4. Western Blot detection of antibodies to HIV. This procedure is performed as previously described [16,17] with special reference to the antibodies for core antigen (p24) reflecting early infection and envelope protein (gp41) as the reliable and confirmatory HIV antibody [26].
5. HIV p24 Antigen capture ELISA. This is performed according to the manufacturer's instructions (Abbott Laboratories).
6. Reverse transcriptase assay. This is done as described [16] with minor modifications [60,58]: instead of an overnight polyethylene glycol (PEG) precipitation of virus at 4°C, virus is pelleted during a two hour centrifugation at 13,000rcf, at 4°C, in a sealed centrifuge. 1.4ml volumes of cell-free media are centrifuged, 24 tubes per spin. Since the PEG concentration technique results in varying degrees of inhibition of p24 Antigen capture ELISA [61], centrifugation offers an efficient means of producing antigen preparations for both reverse transcriptase and antigen capture assays.
7. HIV titration syncytial assay. This assay is based on visual inspection of syncytia (HIV infected multinucleate giant MT2 cells) production on microtiter plates and is performed as previously described [92,93,94]. Cells are exposed to HIV preparations in triplicate and virus is diluted serially ten-fold as in other virus titration procedures. Reed-Muench method is used to calculate the TCID-50 dose. Data is obtained within 7 days and MT2 cells are exposed to DEAE-dextran or polybrene to optimize the yield of syncytia.

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ADDENDUM 1 Tables and Figures from Work Accomplished

Table 1 A
DEMOGRAPHICS
FOR ALL PATIENTS IN BASELINE DATA BASE AND FOR SUBGROUPS ASP†, ARC and AIDS

(UNPUBLISHED DATA - December 1988)

VARIABLE	ALL	ASP	ARC	AIDS	P-value
Number	137	67	60	10	
Age (years)	37.1	35.1	38.9	38.9	.10*
(standard deviation)	(10.2)	(9.5)	(11.3)	(6.2)	
Range (years)	21-72	21-61	25-72	33-51	
Education (years)	14.5	15.1	14.1	13.7	.11*
(standard deviation)	(2.9)	(2.6)	(3.2)	(2.2)	
Range (years)	8-23	9-23	8-22	10-16	
Gender (percent males)	98	97	100	90	.11**
Source (percent)					
Community/Advertisement	43	73	17	10	
Private MDs	10	3	13.6	40	
UCLA ACTG††	23	17	30.4	20	
VA 298 protocol	10	0	22	0	
VA other	11	5	15	30	
APLA††	2	2	1.7	0	
Risk Factors (percent)					
Homosexual or bisexual	82	89	73	90	
Contaminated IV needle	2	2	3	0	
Both 1 & 2 above possible	11	3	22	0	
Heterosexual contact	3	3	2	10	
Transfusion recipient	1	2	0	0	
Artificial insemination	1	1	0	0	
History Drug Use (percent)					
one drug	22	21	26	0	
multiple drugs	24	22	20	29	
History Neuro Problems (%)	28	30	28	14	
History Psych Problems (%)	35	30	37	49	

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE for ASP vs ARC

** Chi-Square

†† ACTG = AIDS Clinical Trials Group
APLA=AIDS Project Los Angeles

Table 1 B
DEMOGRAPHICS
FOR PATIENTS with CSF EXAMINATIONS
AND FOR SUBGROUPS ASP†, ARC and AIDS

(UNPUBLISHED DATA - December 1988)

VARIABLE	ALL	ASP	ARC	AIDS	P-value
Number	102	40	53	9	
Age (years)	37.6	36.6	38.5	39.8	.61*
(standard deviation)	(10.4)	(10.2)	(11.0)	(7.2)	
Range (years)	23-72	23-61	25-72	33-51	
Education (years)	14.7	15.5	14.3	13.6	.08*
(standard deviation)	(2.9)	(2.6)	(3.1)	(2.3)	
Range (years)	8-23	12-23	8-22	10-16	
Gender (percent males)	97	96	100	89	.12**
History Drug Use (percent)					
one drug	21	16	28	0	
multiple drugs	23	19	28	17	
History Neuro Problems (%)	23	27	29	14	
History Psych Problems (%)	34	31	37	40	

Table 2 A
CLINICAL MEDICAL FINDINGS
for SUBGROUPS ASP†, ARC and AIDS

(UNPUBLISHED DATA - December 1988)

VARIABLE	ASP	ARC	AIDS	P-value
Karnofsky Score (0-100)	98.8	87.3	68.0	.0001*
standard deviation	4.1	11.4	17.5	
N	62	55	10	
ARC SYMPTOMS (total number)	0	2.4	3.7	.0001*
standard deviation	0	1.5	1.6	
N	63	56	9	
History (HX) or have #yes/total (%) **Oral Hairy Leukoplakia**	0/69 (0)	7/56 (12)	5/10 (50)	.0001**
HX or have Shingles	0/69 (0)	16/56 (28)	4/10 (40)	.0001**
HX or have Oral Herpes SV	27/67 (40)	22/51 (43)	7/10 (70)	.18
HX or have Genital/Anal Herpes	14/68 (20)	14/52 (27)	4/10 (40)	.15
HX or have CMV disease	0/68 (0)	0/56 (0)	1/10 (10)	

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE for ASP vs ARC

** Chi-Square

Table 2 B
CLINICAL NEUROLOGIC FINDINGS for SUBGROUPS ASP†, ARC and AIDS

(UNPUBLISHED DATA - December 1988)

NEUROLOGIC FINDINGS†	ASP	ARC	AIDS	P value	Scheffe P<0.05
GENERAL MOTOR (0-19)	.2	1.9	1.6	.0001*	ASP<ARC
standard deviation	.61	2.5	1.1		
N	66	52	7		
MOVEMENT DISORDER (0-12)	.1	.4	.4	.02*	ASP<ARC
standard deviation	.6	2.5	1.1		
N	66	52	8		
GLOBAL MOTOR SCORE (0-3)	.1	.4	.8	.0001*	ASP<ARC
standard deviation	.2	.6	.5		ASP<AIDS
N	66	54	8		
ABNORMAL REFLEXES (0-8)	.3	.7	1.4	.0002*	ASP<ARC
standard deviation	.6	.9	1.3		ASP<AIDS
N	66	54	7		
CRANIAL NERVES (range 0-11)	.35	.72	.14	.09*	
standard deviation	.75	1.29	.38		
N	66	54	7		
ABNORMAL SENSORY (0-5)	.3	1.0	1.3	.0001*	ASP<ARC
standard deviation	.5	1.0	1.4		ASP<AIDS
N	66	52	7		
PERIPHERAL NEUROPATHY (0-3)	.2	.6	.8	.0001*	ASP<ARC
standard deviation	.4	.5	.5		ASP<AIDS
N	66	54	8		
DEMENTIA GLOBAL SCORE (0-3)	.2	.7	.5	.0001*	ASP<ARC
standard deviation	.4	.8	.8		
N	66	54	8		
GLOBAL RATINGS #yes/total (%yes)					
NEURO POSITIVE	16/65 (25)	37/52 (71)	9/9 (100)	.0001**	
DEMENTIA (mild, mod or sev)	9/66 (14)	30/54 (56)	3/8 (38)	.0001**	
GLOBAL MOTOR	4/66 (6)	21/54 (39)	6/8 (75)	.0001**	
PERIPHERAL NEUROPATHY	10/66 (15)	30/54 (56)	6/8 (75)	.0001**	

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

†† See Methods Section for defintions; Neurologic Rating Scale from ATEU 005 Study

*1-WAY ANALYSIS OF VARIANCE for ASP vs ARC vs AIDS

** Chi-Square

Table 3 A
CSF and BLOOD FINDINGS RELATED TO HIV STATUS
FREQUENCY OF ABNORMAL FINDINGS
(Unpublished data December 1988)

	ASP†	ARC	AIDS
CEREBROSPINAL FLUID MEASURES			
WBC ≥ 5 cmm	6/39 (15)*	8/52 (15)	1/7 (14)
Intra-Blood Brain Barrier Albumin Leakage ≥ 75 mg/day	10/40 (25)	17/53 (32)	4/9 (44)
Intra-Blood Brain Barrier IgG Synthesis Rate ≥ 3.0 mg/day	29/40 (73)	42/53 (79)	4/9 (44)
IgG Index ≥ 0.7	29/40 (73)	38/53 (72)	4/9 (44)
Unique CSF IgG Bands	22/38 (58)	31/52 (60)	4/9 (44)
IgG Bands More Intense in CSF than in Serum	23/38 (61)	28/52 (54)	3/9 (33)
Abnormal CSF (Rate or Bands)	**35/40 (88)**	**49/53 (92)**	**5/9 (56)**
HIV culture	4/37 (11)	15/47 (32)	2/7 (28)
HIV p24 antigen (≥10 pg/ml)	12/36 (33)	21/51 (41)	4/7 (57)
BLOOD MEASURES			
HIV culture	17/37 (46)	27/46 (59)	7/9 (78)
HIV p24 antigen (≥ 20 pg/ml)	6/39 (15)	12/46 (26)	2/9 (22)
HIV p17 antibody (WB)**	24/39 (62)	30/51 (59)	3/8 (38)
HIV p24 antibody (WB)	29/38 (76)	30/51 (59)	5/8 (62)
HIV p32 antibody (WB)	9/39 (23)	6/51 (12)	3/9 (33)
HIV gp41 antibody (WB)	39/39 (100)	51/51 (100)	9/9 (100)
HIV p51 antibody (WB)	38/39 (97)	46/51 (90)	8/9 (90)
HIV p55 antibody (WB)	38/39 (97)	44/51 (86)	8/9 (90)
HIV p61 antibody (WB)	37/39 (97)	43/51 (84)	9/9 (100)
HIV p70 antibody (WB)	37/39 (97)	45/51 (88)	7/9 (78)
HIV gp120 antibody (WB)	37/39 (97)	44/51 (86)	8/9 (90)
T4 Lymphocytes < 430 cmm	18/39 (46)	37/49 (76)	6/6 (100)
T4:T8 ratio < 1.0	32/38 (84)	48/49 (98)	7/8 (88)
Abnormal Serum IgG Bands	20/38 (53)	25/53 (47)	2/9 (22)

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* #abnormal/total# (% Abnormal)

** WB : Western Blot

Table 3 B
CEREBROSPINAL FLUID FINDINGS for SUBGROUPS ASP†, ARC and AIDS
MEANS AND STANDARD DEVIATIONS
(UNPUBLISHED DATA - December 1988)

CSF MEASURE	ASP	ARC	AIDS	P value
WBC (cmm)	2.8	3.5	8.1	.23*
standard deviation	3.5	7.0	19.8	
N	39	52	7	
I-BBB Albumin Leakage (mg/day)	48.5	50.5	78.7	.26*
standard deviation	48.1	46.2	79.1	
N	40	53	9	
I-BBB IgG Synthesis Rate (mg/day)	18.0	22.5	17.8	.75*
standard deviation	28.1	32.9	25.6	
N	40	53	9	
IgG Index (ratio)	.89	.94	.71	.32*
standard deviation	.4	.5	.2	
N	40	53	9	
UNIQUE CSF IgG BANDS (#)	2.4	2.1	2.3	.89*
standard deviation	3.0	2.3	4.9	
N	38	52	9	
IgG BANDS MORE INTENSE IN CSF THAN IN SERUM (#)	2.5	2.9	1.8	.71*
standard deviation	3.3	4.2	3.5	
N	38	52	9	
HIV p24 ANTIGEN LEVEL (pg/ml)	5.4	12.1	9.8	.54*
standard deviation	6.3	36.5	5.1	
N	35	50	7	

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE for ASP vs ARC

Table 3 C
CSF and BLOOD FINDINGS
RELATED TO NEUROLOGIC ABNORMALITIES
FREQUENCY OF ABNORMAL FINDINGS
(Unpublished data December 1988)

	NEURO NEG		NEURO POS	
CEREBROSPINAL FLUID MEASURES				
WBC ≥ 5 cmm	04/41	(10)*	11/49	(22)
Intra-Blood Brain Barrier Albumin Leakage ≥ 75 mg/day	08/42	(19)	19/50	(38)
Intra-Blood Brain Barrier IgG Synthesis Rate ≥ 3.0 mg/day	30/42	(71)	38/50	(76)
IgG Index ≥ 0.7	29/42	(69)	36/50	(72)
Unique CSF IgG Bands ≥0	20/40	(50)	29/49	(59)
IgG Bands More Intense in CSF than in Serum ≥0	22/40	(55)	25/49	(51)
Abnormal CSF (Rate or Bands)	**35/42**	**(83)**	**44/50**	**(88)**
HIV culture	8/40	(20)	8/41	(20)
HIV p24 antigen (≥10 pg/ml)	10/39	(26)	20/45	(44)
BLOOD MEASURES				
HIV culture	20/41	(49)	27/42	(64)
HIV p24 antigen (≥20 pg/ml)	5/41	(12)	11/44	(25)
HIV p17 antibody (WB)**	24/39	(62)	26/46	(56)
HIV p24 antibody (WB)	27/41	(66)	33/46	(72)
HIV p32 antibody (WB)	30/39	(77)	41/47	(87)
HIV gp41 antibody (WB)	42/42	(100)	47/47	(100)
HIV p51 antibody (WB)	37/39	(95)	43/47	(91)
HIV p55 antibody (WB)	36/39	(92)	42/47	(89)
HIV p61 antibody (WB)	35/39	(63)	42/47	(89)
HIV p70 antibody (WB)	36/39	(92)	40/47	(85)
HIV gp120 antibody (WB)	36/39	(92)	40/47	(85)
T4 Lymphocytes < 430 cmm	17/39	(44)	32/43	(74)
T4:T8 Ratio < 1.0	32/38	(84)	40/42	(95)
Abnormal Serum IgG Bands	18/40	(45)	24/49	(49)

* #abnormal/total# (% Abnormal)
** WB : Western Blot

Table 3 D
CSF and BLOOD FINDINGS
RELATED TO NEUROLOGIC ABNORMALITIES
MEANS AND STANDARD DEVIATIONS
(Unpublished data December 1988)

	NEURO NEG n=(42)	NEURO POS n=(53)	p-value*
CEREBROSPINAL FLUID MEASURES			
WBC (cmm)	2.3	4.8	.11
Intra-Blood Brain Barrier Albumin Leakage (mg/day)	39.3	53.1	.26
Intra-Blood Brain Barrier IgG Synthesis Rate (mg/day)	11.1	29.3	**.003**
IgG Index (ratio)	.87	.96	.30
Unique CSF IgG Bands (#)	1.8	2.2	.52
IgG Bands More Intense in CSF than in Serum (#)	1.8	2.8	.17
HIV p24 antigen level (pg/ml)	4.4	13.0	.16
BLOOD MEASURES			
HIV p24 antigen level (pg/ml)	11.6	44.6	**.03**
T4 Lymphocytes (cmm)	466	334	**.004**
T4:T8 Ratio	.74	.46	**.004**
Abnormal Serum IgG Bands (#)	2.0	2.5	.49

* 1-WAY ANALYSIS OF VARIANCE

Table 3 E
CSF and BLOOD FINDINGS RELATED TO THE PRESENCE OR ABSENCE OF CNS MOTOR SIGNS

(Unpublished data December 1988)

	WITH SIGNS N=(31)	WITHOUT SIGNS N=(64)	p-value†
CEREBROSPINAL FLUID MEASURES			
WBC (cmm)	7.5	2.3	**.003**
Intra-Blood Brain Barrier Albumin Leakage (mg/day)	55.4	44.5	.42
Intra-Blood Brain Barrier IgG Synthesis Rate (mg/day)	27.4	18.1	.19
IgG Index (ratio)	.95	.89	.53
Unique CSF IgG Bands (#)	2.1	2.3	.72
IgG Bands More Intense in CSF than in Serum (#)	3.6	2.3	.14
HIV culture	5/21(23.8)*	12/54(18.8)	.62
HIV p24 antigen level (pg/ml)	21.3	5.4	**.01**
BLOOD MEASURES			
HIV culture	14/22(63.6)	34/65(52.3)	.36
HIV p24 antigen levels (pg/ml)	52.9	25.4	.12
HIV p24 antibody (WB)**	15/23(65.2)	47/68(69.1)	.73
HIV gp41 antibody (WB)	23/23(100)	68/68(100)	
T4 Lymphocytes (cmm)	344.3	405.2	.22
T4:T8 Ratio	.4	.6	.13
Abnormal Serum IgG Bands	3.1	2.4	.44

† 1-WAY ANALYSIS OF VARIANCE

* #abnormal/total# (% Abnormal)

** WB : Western Blot

Table 3 F
CSF and BLOOD FINDINGS RELATED TO THE PRESENCE OR ABSENCE OF DEMENTIA

(Unpublished data December1988)

	DEMENTED N=(33)	NOT DEMENTED N=(51)	p-value†
CEREBROSPINAL FLUID MEASURES			
WBC (cmm)	4.2	3.5	.67
Intra-Blood Brain Barrier Albumin Leakage (mg/day)	48.2	41.5	.56
Intra-Blood Brain Barrier IgG Synthesis Rate (mg/day)	27.0	15.4	.08
IgG Index (ratio)	1.0	.9	.35
Unique CSF IgG Bands (#)	2.0	2.1	.94
IgG Bands More Intense in CSF than in Serum (#)	3.0	2.3	.40
HIV culture	7/31(22.6)*	10/49(20.4)	.82
HIV p24 antigen levels (pg/ml)	15.9	5.1	.08
BLOOD MEASURES			
HIV culture	22/31(71)	24/50(48)	**.04**
HIV p24 antigen levels (pg/ml)	49.2	22.7	.11
HIV p24 antibody (WB)**	22/33(66.7)	35/51(68.6)	.85
HIV gp41 antibody (WB)	33/33(100)	51/51(100)	
T4 Lymphocytes (cmm)	369.7	409.6	.39
T4:T8 Ratio	.4	.6	**.05**
Abnormal Serum IgG Bands	2.2	2.8	.51

† 1-WAY ANALYSIS OF VARIANCE

* #abnormal/total# (% Abnormal)

** WB : Western Blot

Table 4 A
NEUROPSYCHOLOGICAL TEST RESULTS FOR ASP† versus ARC

(UNPUBLISHED DATA - December 1988)

TEST	ASP N=57	ARC N=36	P-value*
VERBAL			
Vocabulary (# points)	55.1	53.7	.50
Verbal Fluency (#words in 180 sec)	43.9	44.2	.92
Verbal Learning & Memory (Rey AVLT)			
Trial 1-5 (avg. # correct)	10.6	10.2	.43
Trial 6 -recall (# correct)	11.1	10.1	.12
VISUOSPATIAL			
Block Design (point sum)	36.1	32.8	.14
VISUOMOTOR TRACKING			
Trails A (# secs)	25.9	29.0	.14
VISUOMOTOR CONCEPTUALIZATION			
Trails B (# secs)	58.5	68.8	.07
Digit-Symbol Substitution (#correct/90')	64.3	58.5	**.03**
VISUAL ATTENTION - IMMEDIATE MEMORY			
Benton Visual Retention Test			
# correct out of 10	7.7	7.0	**.05**
# errors	3.3	4.9	**.01**
AUDITORY ATTENTION - IMMEDIATE MEMORY			
Digit Span (forward+ backward # recalled)	14.5	11.5	.12
MANUAL DEXTERITY			
Grooved Pegboard -Dom(#secs to complete)	67.3	76.7	**.0001**
Nondom	73.7	84.2	**.002**
AGE	35.3	35.8	.80
EDUCATION (yrs)	17.3	15.2	.40

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE

Table 4 B
NEUROPSYCHOLOGICAL TEST RESULTS RELATED TO NEUROLOGICAL ABNORMALITIES

(UNPUBLISHED DATA - December 1988)

TEST	NEURO POS N=37	NEURO NEG N=56	P-value*
VERBAL			
Vocabulary (# points)	53.9	55.0	.57
Verbal Fluency (#words in 180 sec)	40.1	46.7	**.01**
Verbal Learning & Memory (Rey AVLT)			
Trial 1-5 (avg. # correct)	9.9	10.8	**.03**
Trial 6 -recall (# correct)	9.7	11.4	**.008**
VISUOSPATIAL			
Block Design (point sum)	33.1	35.9	.21
VISUOMOTOR TRACKING			
Trails A (# secs)	30.3	25.0	**.008**
VISUOMOTOR CONCEPTUALIZATION			
Trails B (# secs)	72.2	56.1	**.003**
Digit-Symbol Substitution (#correct/90')	56.2	65.9	**.0002**
VISUAL ATTENTION - IMMEDIATE MEMORY			
Benton Visual Retention Test			
# correct out of 10	6.7	7.9	**.0003**
# errors	5.2	3.0	**.0002**
AUDITORY ATTENTION - IMMEDIATE MEMORY			
Digit Span (forward+ backward # recalled)	12.2	14.1	.33
MANUAL DEXTERITY			
Grooved Pegboard -Dom(#secs to complete)	75.9	67.5	**.0002**
Nondom	84.0	73.5	**.001**
AGE	38.8	33.2	**.003**
EDUCATION (yrs)	15.4	15.2	.66

* 1-WAY ANALYSIS OF VARIANCE

Table 4 C
NEUROPSYCHOLOGICAL TEST RESULTS BY T4 GROUPINGS

(UNPUBLISHED DATA - December 6, 1988)

	T4 Lymphocyte cell count			
TEST	0-249 N=28	250-429 N=29	≥430 N=34	P-value*
VERBAL				
Vocabulary (# points)	56.6	51.9	54.3	.15
Verbal Fluency (# words/180")	45.0	42.6	43.8	.79
Verbal Learning & Memory (Rey AVLT)				
Trial 1-5 (avg. # correct)	9.7	10.0	10.7	.09
Trial 6 -recall (# correct)	9.2	10.5	10.7	.13
VISUOSPATIAL				
Block Design (point sum)	32.5	32.8	37.2	.14
VISUOMOTOR TRACKING				
Trails A (# secs)	29.8	28.5	23.9	**.04**
VISUOMOTOR CONCEPTUALIZATION				
Trails B (# secs)	68.7	72.4	56.3	.07
Digit-Symbol Substitution (#correct/90')	57.5	58.5	65.7	**.02**
VISUAL ATTENTION - IMMEDIATE MEMORY				
Benton Visual Retention Test				
# correct out of 10	7.0	6.9	7.7	.10
# errors	4.7	4.8	3.2	**.04**
AUDITORY ATTENTION - IMMEDIATE MEMORY				
Digit Span (forward+ backward # recalled)	12.1	11.7	15.3	.21
MANUAL DEXTERITY				
Grooved Pegboard -Dom(#secs to complete)	77.3	76.9	66.5	**.0005**
Nondom	80.6	83.8	71.9	**.01**
AGE	41.5	35.0	35.7	**.02**
EDUCATION (yrs)	15.0	15.5	18.4	.46

* 1-WAY ANALYSIS OF VARIANCE

Table 5 A
COMPUTERIZED MEMORY EVALUATION FOR ASP† and ARC

(UNPUBLISHED DATA - December 1988)

TEST	ASP N=44	ARC N=30	P-value*
NAME-FACE ASSOCIATION			
6 people (# recalled)	2.9	2.3	.12
14 people-Trial 1	5.1	4.0	.12
14 people-Trial 2	10.1	8.6	**.05**
14 people-Trial 3	12.6	11.2	**.02**
14 people-30' delay	12.0	10.7	.08
DIVIDED ATTENTION			
Trial 1-Lift time (in msec)	364.8	418.9	**.009**
Trial 2-Lift time (in msec)	330.7	382.1	**.01**
Trial 1-Memory points	11.1	9.0	.13
Trial 2-Memory points	9.2	9.6	.69
FIRST-LAST NAMES			
Trial 1-(# out of 6 recalled)	1.6	1.4	.49
Trial 2	3.5	2.9	.11
Trial 3	4.0	3.5	.25
Trial 4	4.4	3.9	.21
Trial 5	5.1	4.4	**.05**
TELEPHONE DIALING			
7 digits-no interference (# digits correct)	6.8	6.9	.54
7 digits-before interference	6.8	6.8	.56
7 digits-after interference	6.3	6.5	.29
10 digits-no interference	8.0	7.6	.33
10 digits-before interference	8.1	7.8	.38
10 digits-after interference	6.3	5.8	.32
INCIDENTAL MEMORY			
# citites recalled from Name-Face Assoc	5.2	4.3	.26
MISPLACED OBJECTS			
# of objects found on 1st try (of 20)	13.6	14.3	.46
# of objects found on 2nd try	16.2	16.6	.53
# objects not found	3.8	3.4	.53
AGE	36.0	36.1	.96
EDUCATION (yrs)	18.1	14.9	.29

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE

Table 5 B
COMPUTERIZED MEMORY EVALUATION RELATED TO NEUROLOGICAL ABNORMALITIES

(UNPUBLISHED DATA - December 1988)

TEST	NEURO POS N=30	NEURO NEG N=44	P-value*
NAME-FACE ASSOCIATION			
6 people (# recalled)	2.4	2.8	.34
14 people-Trial 1	3.9	5.2	.06
14 people-Trial 2	8.3	10.3	**.01**
14 people-Trial 3	10.8	12.9	**.0005**
14 people-30' delay	10.1	12.5	**.0006**
DIVIDED ATTENTION			
Trial 1-Lift time (in msec)	423.6	361.6	**.002**
Trial 2-Lift time (in msec)	379.5	332.5	**.02**
Trial 1-Memory points	9.3	10.8	.25
Trial 2-Memory points	9.2	9.5	.81
FIRST-LAST NAMES			
Trial 1-(# out of 6 recalled)	1.2	1.7	.07
Trial 2	2.6	3.7	**.001**
Trial 3	3.3	4.1	**.04**
Trial 4	3.5	4.7	**.003**
Trial 5	4.2	5.3	**.0008**
TELEPHONE DIALING WITH INTERFERENCE			
7 digits-no interference (# digits correct)	6.8	6.8	.17
7 digits-before interference	6.9	6.7	.24
7 digits-after interference	6.4	6.4	.85
10 digits-no interference	7.6	8.0	.24
10 digits-before interference	7.8	8.1	.47
10 digits-after interference	5.7	6.3	.26
INCIDENTAL MEMORY			
# cities recalled from Name-Face Assoc	3.5	5.7	**.006**
MISPLACED OBJECTS			
# of objects found on 1st try (of 20)	13.5	14.7	.44
# of objects found on 2nd try	16.2	16.5	.66
# objects not found	3.8	3.5	.66
AGE	44.0	33.4	**.002**
EDUCATION (yrs)	15.2	15.2	.99

* 1-WAY ANALYSIS OF VARIANCE

Table 5 C
FREQUENCY OF NEUROPSYCHOLOGICAL ABNORMALITIES FOR ASP† and ARC/AIDS GROUPS

(UNPUBLISHED DATA - December 1988)

	ASP N=41	ARC/AIDS N=30
NORMAL•	40(97.6)	22(73.3)
ABNORMAL••	1(2.4)	8(26.7)

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

Chi-Square: p=.002

•=≤1 test score -2 SD below the mean
••=≥2 test scores -2 SD below the mean

FREQUENCY OF COMPUTERIZED MEMORY TEST ABNORMALITIES FOR ASP† and ARC/AIDS GROUPS

	ASP N=45	ARC/AIDS N=33
NORMAL•	34(75.6)	16(48.5)
ABNORMAL••	11(24.4)	17(51.5)

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

Chi-Square: p=.01

•=≤1 test score -2 SD below the mean
••=≥2 test scores -2 SD below the mean

Table 6 A
NEURO-PERFORMANCE TESTS FOR ASP†, ARC & AIDS
Upper Extremity

(Unpublished data December, 1988)

UPPER EXTREMITY TESTS	ASP (n=53)	ARC (n=34)	AIDS (n=6)	P-value*
SPEED				
Hand Tapping - Dom	61.8	59.7	53.5	.09
Hand Tapping - Nondom	60.0	55.6	52.4	.01
REACTION TIME				
Simple: Dom(in msecs)	227.0	262.7	261.2	.01
Nondom (in msecs)	215.4	254.9	248.1	.007
Multiple: Dom(in msecs)	327.7	388.1	341.6	.001
Nondom (in msecs)	310.0	352.5	348.0	.003
DEXTERITY & COORDINATION				
Large Peg Rotation (in sec)	10.8	9.7	10.4	.01
Purdue Pegboard:				
Dom(#/30 sec)	15.1	13.5	14.2	.0007
Nondom	15.7	14.2	13.8	.0009
Alternating Tapping:				
Dom(# hits)	8.6	8.2	7.8	.17
Nondom	8.0	7.4	7.5	.18
STEADINESS				
Resting - Vertical: Dom	0.26	0.37	0.45	.59
Nondom	0.23	0.34	0.33	.57
Sustention - Vertical: Dom	1.8	2.4	2.3	.004
Nondom	1.9	2.4	1.8	.03
TRACKING - RANDOM 2-dimensional				
Vertical error: Dom	0.9	1.2	0.8	.004
Vertical error: Nondom	0.9	1.1	0.9	.001
STRENGTH (lbs)				
Grip - Dom	75.9	74.7	71.1	.76
Grip - Nondom.	71.2	71.0	65.4	.71
Shoulder - Dom	16.2	15.8	16.3	.96
Shoulder - Nondom.	15.6	15.6	14.0	.82
VIBRATION THRESHOLD				
Finger Pad - Dom	0.7	0.8	0.7	.87
Finger Pad - Nondom	0.6	0.6	0.9	.69
AGE	36.1	36.8	40.0	.65
EDUCATION (yrs)	17.6	14.7	14.8	.50

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE

Addendum

Table 6 B
NEURO-PERFORMANCE TESTS RELATED TO NEUROLOGICAL ABNORMALITIES
Upper Extremity

(Unpublished data December 1988)

UPPER EXTREMITY TESTS	NEURO POS (n=36)	NEURO NEG (n=55)	P-value*
SPEED			
Hand Tapping - Dom	58.3	62.9	.03
Hand Tapping - Nondom	55.9	60.8	.007
REACTION TIME			
Simple: Dom (in msecs)	261.2	225.0	.003
Nondom (in msecs)	253.2	213.0	.001
Multiple: Dom (in msecs)	370.5	321.8	.002
Nondom (in msecs)	346.5	303.9	.0007
DEXTERITY & COORDINATION			
Large Peg Rotation (in sec)	10.2	10.7	.11
Purdue Pegboard: Dom (#/30 sec)	13.8	15.2	.0003
Nondom	14.2	15.9	.0001
Alternating Tapping: Dom (# hits)	8.1	8.9	.01
Nondom	7.3	8.4	.0006
STEADINESS			
Resting - Vertical: Dom	0.30	0.30	.95
Nondom	0.30	0.20	.83
Sustention - Vertical: Dom	2.1	1.9	.40
Nondom	2.1	2.0	.32
TRACKING - RANDOM 2-dimensional			
Vertical error: Dom	1.1	0.9	.06
Vertical error: Nondom	0.9	1.0	.04
STRENGTH (lbs)			
Grip - Dom	71.6	77.8	.08
Grip - Nondom.	67.1	73.7	.07
Shoulder - Dom	15.3	16.4	.36
Shoulder - Nondom.	14.3	16.0	.17
VIBRATION THRESHOLD			
Finger Pad - Dom	0.9	0.6	.12
Finger Pad - Nondom	0.7	0.5	.24
AGE	39.1	33.9	.006
EDUCATION (yrs)	15.2	17.4	.38

* 1-Way ANALYSIS OF VARIANCE

Table 6 C
NEURO-PERFORMANCE TESTS FOR T4-CELL GROUPS
Upper Extremity

(Unpublished data December 1988)

UPPER EXTREMITY TESTS	0-249	250-499 (n=25)	≥500 (n=25)	P-value* (n=23)
SPEED				
Hand Tapping - Dom	60.3	60.2	60.7	.98
Hand Tapping - Nondom	56.9	56.9	59.4	.47
REACTION TIME				
Simple: Dom (in msecs)	251.1	255.0	211.9	**.01**
Nondom (in msecs)	237.2	240.2	201.7	**.02**
Multiple: Dom (in msecs)	365.5	356.5	325.0	.17
Nondom (in msecs)	329.7	348.3	306.6	**.04**
DEXTERITY & COORDINATION				
Large Peg Rotation (in sec)	10.1	10.3	10.6	.50
Purdue Pegboard: Dom (#/30 sec)	13.6	14.4	15.1	**.03**
Nondom	13.8	15.3	15.6	**.003**
Alternating Tapping: Dom (# hits)	7.9	8.3	8.9	.06
Nondom	7.4	7.9	8.0	.24
STEADINESS				
Resting - Vertical: Dom	0.30	0.39	0.25	.68
Nondom	0.14	0.38	0.25	.17
Sustention - Vertical: Dom	2.1	2.0	2.0	.92
Nondom	1.9	2.0	2.1	.83
TRACKING -RANDOM 2-dimensional				
Vertical error: Dom	1.1	1.1	1.0	.53
Vertical error: Nondom	1.1	1.0	0.9	.21
STRENGTH (lbs)				
Grip - Dom	77.7	75.9	72.6	.54
Grip - Nondom.	74.1	71.8	66.4	.26
Shoulder - Dom	17.2	16.3	14.4	.18
Shoulder - Nondom.	16.8	15.3	14.7	.43
VIBRATION THRESHOLD				
Finger Pad - Dom	0.7	0.6	0.7	.94
Finger Pad - Nondom	0.7	0.5	0.7	.78
AGE	42.5	33.8	36.1	**.002**
EDUCATION (yrs)	14.9	15.4	19.9	.29

* 1-Way ANALYSIS OF VARIANCE

Table 7 A
NEURO-PERFORMANCE TESTS FOR ASP†, ARC & AIDS
Lower Extremity

Unpublished data December, 1988

LOWER EXTREMITY TESTS	ASP (n=40)	ARC (n=27)	AIDS (n=6)	P-value*
SPEED				
Foot Tapping - Dom	46.8	42.2	44.7	.03
Foot Tapping - Nondom	43.8	39.4	40.3	**.02**
REACTION TIME				
Simple: Dom (in msecs)	275.5	333.4	373.1	**.001**
Nondom (in msecs)	294.8	327.3	392.3	**.007**
COORDINATION				
Alternating Tapping: Dom (# hits)	11.2	8.0	7.3	.28
Nondom	9.2	8.2	7.1	.05
STRENGTH (lbs)				
Thigh - Dom	26.2	23.3	20.9	.19
Thigh - Nondom	24.4	21.3	18.9	.13
STANDING BALANCE				
Dom. leg-eyes open	28.9	23.5	29.0	**.002**
Nondom. leg-eyes open	29.9	25.0	25.3	**.0007**
Dom. leg-eyes closed	16.8	12.1	13.8	.12
Nondom. leg-eyes closed	17.9	11.1	4.6	**.0006**
TANDEM GAIT				
Steps/sec.	1.8	1.6	1.4	.06
VIBRATION THRESHOLD				
Hallux - Dom	3.3	4.3	7.3	.06
Hallux - Nondom	3.3	20.4	8.8	.40
AGE	36.1	36.8	40.0	.65
EDUCATION (yrs)	17.6	14.7	14.8	.50

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE

Table 7 B
NEURO-PERFORMANCE TESTS RELATED TO NEUROLOGICAL ABNORMALITIES
Lower Extremity

(Unpublished data December 1988)

LOWER EXTREMITY TESTS	NEURO POS (n=35)	NEURO NEG (n=51)	P-value*
SPEED			
Foot Tapping - Dom	43.6	47.1	.03
Foot Tapping - Nondom	40.5	44.3	.02
REACTION TIME			
Simple: Dom (in msecs)	333.5	272.8	.01
Nondom (in msecs)	335.8	290.5	.004
COORDINATION			
Alternating Tapping: Dom (# hits)	8.2	11.2	.19
Nondom	7.8	7.5	.003
STRENGTH (lbs)			
Thigh - Dom	22.2	26.7	.02
Thigh - Nondom.	20.6	24.9	.02
STANDING BALANCE-1 leg(#sec)			
Dom. leg-eyes open	25.4	28.7	.02
Nondom. leg-eyes open	26.9	29.2	.05
Dom. leg-eyes closed	11.2	18.7	.0007
Nondom.leg-eyes closed	10.5	17.8	.0006
TIMED GAIT			
# Steps/sec.	1.5	1.8	.003
VIBRATION THRESHOLD			
Hallux - Dom	4.8	3.0	.05
Hallux - Nondom	19.0	3.1	.20
AGE	35.8	35.5	.91
EDUCATION (yrs)	15.7	14.8	.15

* 1- Way ANALYSIS OF VARIANCE

Table 7 C
NEURO-PERFORMANCE TESTS FOR T4-CELL GROUPS
Lower Extremity

(Unpublished data December 1988)

LOWER EXTREMITY TESTS	0-249	250-499 (n=40)	≥500 (n=27)	P-value* (n=23)
SPEED				
Foot Tapping - Dom	44.2	45.5	44.4	.80
Foot Tapping - Nondom	42.5	41.3	42.5	.75
REACTION TIME				
Simple: Dom (in msecs)	304.6	311.1	288.4	.66
Nondom (in msecs)	319.5	306.3	297.6	.68
COORDINATION				
Alternating Tapping:				
Dom (# hits)	7.9	11.3	9.7	.55
Nondom	7.8	8.5	9.4	.16
STRENGTH (lbs)				
Thigh - Dom	25.0	25.0	24.2	.94
Thigh - Nondom.	24.4	22.6	21.5	.50
STANDING BALANCE				
Dom. leg-eyes open	24.5	26.3	29.3	.08
Nondom. leg-eyes open	25.0	28.5	28.9	.06
Dom. leg-eyes closed	12.1	14.9	17.3	.22
Nondom. leg-eyes closed	11.5	14.1	17.7	.13
TIMED GAIT				
Steps/sec.	1.6	1.6	1.8	.23
VIBRATION THRESHOLD				
Hallux - Dom	5.1	3.4	4.0	.33
Hallux - Nondom	5.7	3.0	26.9	.30
AGE	36.1	36.8	40.0	.65
EDUCATION (yrs)	17.6	14.7	14.8	.50

* 1- Way ANALYSIS OF VARIANCE

Table 8 A
PSYCHOLOGICAL RESULTS FOR ASP†,ARC, and AIDS
(UNPUBLISHED DATA - December 1988)

	ASP N=48	ARC N=36	AIDS N=7	P-value*
BRIEF SYMPTOM INDEX SCALES				
Somatization	0.4	1.2	0.9	.0001
Depression	0.8	1.3	1.4	.03
Anxiety	0.6	1.2	0.9	.01
General Severity Index	0.5	1.2	1.0	.0001
Positive Symptom Total	18.2	31.9	27.6	.0001
BECK DEPRESSION INVENTORY	N=25	N=7	N=1	
Total score	8.7	22.3	16.0	.0002
NEURO-BEHAVIORAL RATING	N=66	N=54	N=8	
Behavioral Disturbances (0-3)	0.3	1.0	0.6	.0001

Table 8 B
RELATED TO NEUROLOGICAL ABNORMALITIES

	NEURO POS N=35	NEURO NEG N=47	P-value*
BRIEF SYMPTOM INDEX SCALES			
Somatization	1.2	0.3	.0001
Depression	1.3	0.8	.008
Anxiety	1.1	0.6	.007
General Severity Index (total score/#questions)	1.1	0.5	.0001
Positive Symptom Total	30.6	18.3	.0001
BECK DEPRESSION INVENTORY	N=8	N=25	
Total score	19.8	9.2	.001

Table 8 C
BY T4 GROUPINGS

	0-249 N=17	250-429 N=24	≥430 N=28	P-value*
BRIEF SYMPTOM INDEX SCALES:				
Somatization	0.8	1.0	0.5	.03
Depression	0.7	1.2	0.9	.25
Anxiety	0.7	1.1	0.8	.36
General Severity Index	0.7	1.0	0.6	.24
Positive Symptom Total	23.2	27.9	20.9	.17
BECK DEPRESSION INVENTORY	N=4	N=8	N=13	
Total Score	11.4	10.1	10.6	.97

† **ASP** = systemically asymptomatic HIV seropositive; **ARC** = AIDS Related Complex; **AIDS** = Acquired Immune Deficiency Syndrome without CNS opportunistic infection; all 3 may include individuals with and without HIV-related neurological disease

* 1-WAY ANALYSIS OF VARIANCE

Addendum

Table 9 A
EVOKED POTENTIAL RESULTS FOR ASP† AND ARC

(Unpublished data December 1988)

EVOKED POTENTIAL TEST	ASP N=30	ARC N=29	P-value*
Visual P100 component(in msecs.) Checkerboard Pattern Shift			
Right eye	109.4	110.8	.50
Left eye	107.8	110.4	.14
Auditory (in msecs.) Target Detection Task			
N1 component	85.8	86.8	.71
P300 component	318.8	313.7	.56

† **ASP** = systemically asymptomatic HIV seropositive;
ARC = AIDS Related Complex;
AIDS = Acquired Immune Deficiency Syndrome without CNS opportunistic infection;
all 3 may include individuals with and without HIV-related neurological disease

* 1-Way Analysis of Variance

Table 9 B
EVOKED POTENTIAL RESULTS RELATED TO NEUROLOGICAL ABNORMALITIES

(Unpublished data December 1988)

TEST	NEURO POS N=22	NEURO NEG N=30	P-value*
Visual P100 component(in msecs.) Checkerboard Pattern Shift			
Right eye	111.3	110.1	.60
Left eye	111.0	107.9	.11
Auditory (in msecs.) Target Detection Task			
N1 component	84.6	86.2	.61
P300 component	322.6	311.9	.25

TABLE 10
CHARACTERISTICS OF THE 19 PATIENTS WITH PAIRED HIV BLOOD AND CSF ISOLATES

Clinical Neuro Status*	Stage (# of Patients)		
	ASP	ARC	AIDS
Presence (+)	1	9	1
Absence (-)	3	5	0
TOTAL	4	14	1

* (+) denotes presence and (-) absence of neurologic disease
ASP, denotes symtemically asymptomatic seropositive individuals

TABLE 11
GROUP SPECIFIC NEUTRALIZATION USING HUMAN SERA

	SERUM YEAR		
Virus-Year	WMJ-84	WMJ-85	AY-85
WMJ-84	0	40*	0
WMJ-85	40	40	0
AY-85	40	0	0

* Dilution of serum that neutralized 90% of 5-50 TCID 50.

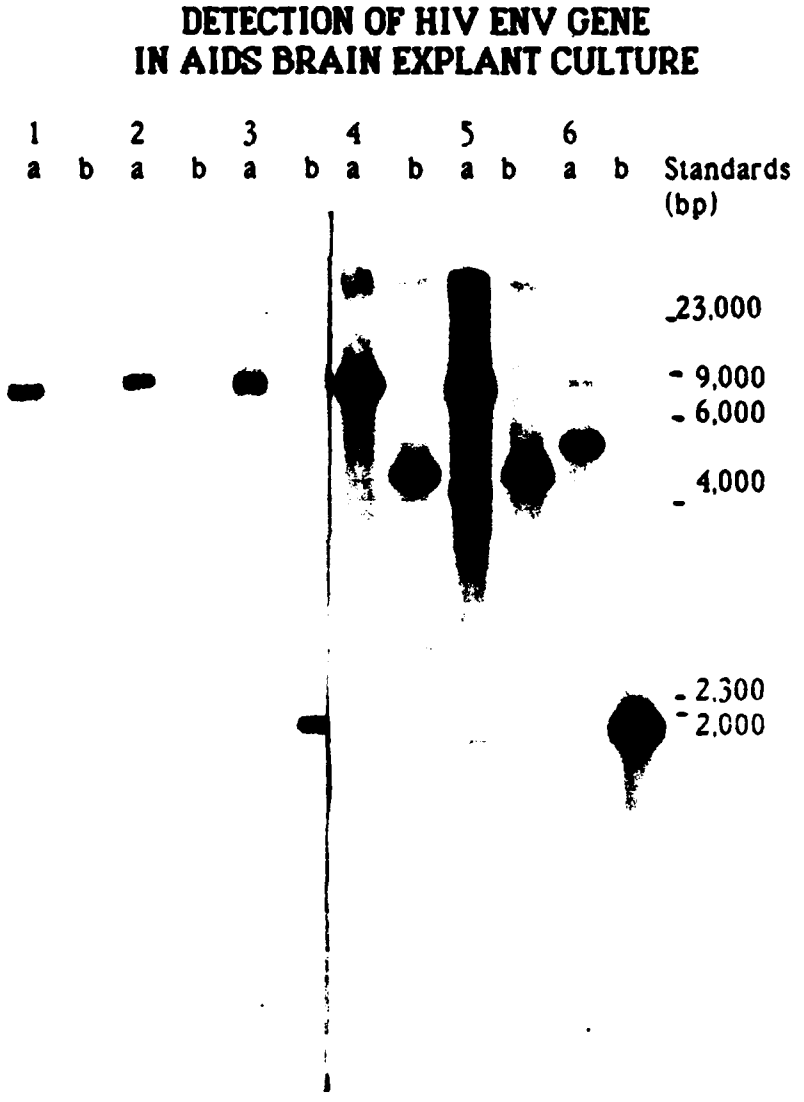
TABLE 12
TYPE SPECIFIC NEUTRALIZATION USING HYPER-IMMUNE GOAT SERA

	SERUM		
Virus-Year	Goat 1	Goat 2	AY-85
WMJ-85	0*	0	640
MPF-85	0	0	640
HTLV-III(III-B)	640	640	160

*Dilution of serum tat reduced TCID 50 by 90%

Addendum

Figure 1: DETECTION OF HIV ENV GENE IN AIDS BRAIN EXPLANT CULTURE.



Addendum

Figure 1 legend: DETECTION OF HIV ENV GENE IN AIDS BRAIN EXPLANT CULTURE.*

High (lanes 1-3) and low (lanes 4-6) molecular weight DNA was prepared using techniques described in the Material and Methods section. A Southern blot was prepared from a 1% agarose gel and hybridized against a ^{32}P-labeled HIV env probe (6.34×10^8 dpm/ug). A 2 ug quantity of DNA was used for each lane. Each "a" lane is the result of digestion with Eco R1 enzyme and each "b" lane is the result of digestion with Hind III enzyme. The DNA in each lane was prepared from the following cultures:
lane 1, AIDS brain culture, 36 days post-explant. Cultures from two wells were combined for the DNA analysis (6-1-34 and 6-1-35). Culture supernatants from this brain did not yield reverse transcriptase activity and four wells of thirty showed marginal levels (39-94 pg/ml) of HIV p24 antigen. These findings demonstrate the sensitivity of the Southern Blot technique; the specificity is being examined by the use of a different strain of HIV env sequence as probe and by increasing the stringency of the washes;
lane 2, MT2 cells infected with an HIV virus produced by COS cells previously transfected with pHXBCat and LTRenv;
lane 3, MT2 cells infected with an HIV virus produced by COS cells previously transfected with pHXB2gbt;
lane 4, COS cells transfected with HS-LTRenv and pHXBCat. The heat shock promoter was activated by heating the cell extracts at 42°C for 30 minutes. The env gene was cloned from an HIV isolate provided by Dr. Wade Parks (Cancer Center, University of Miami Medical School, Miami, Florida).
lane 5, COS cells transfected with HS-LTRenv and pHXBCat. The heat shock promoter was similarly activated by heating the cell extracts at 42°C for 30 minutes. The env gene was cloned from another HIV isolate also provided by Dr. Wade Parks;
lane 6, COS cells transfected with pHXB2gpt, an expression clone of HIV_B, originally provided by Dr. R. C. Gallo (NCI, NIH).
*These studies were performed in conjunction with Dr. Paul Schiller and Professor Richard Voellmy, Department of Molecular Biology, University of Miami Medical School.

Addendum